Chapter 3

Fungi In Soil

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I. INTRODUCTION

Fifty years ago, Waksman (1916a) raised the question whether soil is the home of an indigenous mycoflora, or merely a resting place for fungal spores floating in the atmosphere. He and subsequent workers have indicated that many fungi grow and reproduce in soil; nevertheless soil is also undoubtedly a "sink" for a wide range of organisms from other habitats. As pointed out by Harley (1960), the term *soil fungi* has no precise meaning. It is applied to the heterogeneous collection of fungi which may be isolated from soil, or which have been observed to exist in some form in soil. With some fungi, the soil phase appears to be little more than a resting spore; other organisms appear confined to soil and complete their life cycle there. In a broad sense the organic layers on the surface of mineral soil are here included as part of the soil complex.

The range of fungi known to occur in soil is very wide, from chytrids to agarics, from saprophytes to root parasites, from parasites of amoebae to parasites of man. Interest in fungi occurring in soil has been great and Cooke (1958) considers that soil has probably been studied more extensively than any other natural habitat of fungi. This is partly because of the importance of

fungi as plant pathogens, partly because of their importance in the decomposition of plant and animal residues, and partly through interest in mycorrhiza and the rhizosphere. Many workers have been interested in the ecology of fungi in soil; others have considered soil more as a reservoir for interesting or useful organisms in studies ranging from taxonomy to search for fungi producing antibiotics. This diversity of interest has meant that data on the ecology of fungi in soil are both scattered and in some respects surprisingly fragmentary. Chesters (1949) remarked that so far only a very indistinct picture has been obtained of the fungus at work.

Although knowledge of fungal fructifications occurring on soil is ancient, Adametz in 1886 is considered to have been the first person to isolate fungi from soil. A more detailed study was made by Oudemans and Koning (1902), who inoculated plates of wort agar or wort gelatin with aqueous suspensions, obtained by pulverizing in sterile water, fragments of organic matter extracted from soil. Forty-five species of fungi were identified. Further early studies were those of Hagem (1907, 1910) on the Mucorales in Norwegian soils; Lendner (1908), who studied Mucorales in Switzerland; Dale (1912, 1914), who isolated over 100 fungi from sandy, chalk, peat and black earth soils in England; Beckwith (1911), who investigated some "wheat-sick" soils of North Dakota; and Jensen (1912), who studied the fungal flora of several soils in the U.S.A. The majority of early investigations fall into one or more of three classes: purely systematic studies, physiological or biochemical research, and quantitative studies involving numerical estimates of the fungal flora of soils.

One of the first soilborne diseases to be ascribed to the activity of a parasitic fungus was stem canker of potatoes, which Kühn in 1858 showed to be due to infection by *Rhizoctonia solani*. Other early-ascribed diseases include wilt of potatoes, caused by *Verticillium albo-atrum*, clubroot of crucifers, caused by *Plasmodiophora brassicae*, "take-all" and foot rot of wheat, caused by *Ophiobolus graminis*, and root diseases of forest trees, caused by *Armillaria mellea* (Garrett, 1944). Early work was mainly concerned with elucidation of the cause of various root diseases, but later it was realized that the soil environment exercises a profound effect upon the development of most soilborne pathogens. It was also realized that the soil environment contains biological as well as chemical and physical factors, and that the development of a root disease might be affected not only by the parasite concerned but also by other organisms present. This led many plant pathologists to microbiological studies of soil for, as Garrett (1955) has said, "it is their saprophytic behaviour in the soil that is still the hidden phase in the life cycle of root-infecting fungi."

In 1885, Frank gave the name "mycorrhiza" to the composite fungus-root organ of the Cupuliferae. Associations between fungal hyphae and roots were described in other arborescent angiosperms, and mycorrhiza of the same type were found in many conifers, especially the Pinaceae. They are characterized by the presence of a complete sheath of fungal tissue which encloses the terminal rootlets of the root system. Further studies showed that the roots of

many other plants, previously believed to be free from fungal infection, were also colonized by hyphae. In these no external fungal sheath was found, but the mycelium penetrated and ramified through the cortex. Two types of mycorrhiza were therefore recognized: ectotrophic mycorrhiza having an external fungal sheath, and endotrophic mycorrhiza lacking a sheath. The mycorrhizal condition is found in some members of all higher plant phyla; it appears at least as usual amongst seed plants as the uninfected state (Harley, 1959). The majority of the fungi which form ectotrophic mycorrhiza belong to the Basidiomycetes; endotrophic mycorrhiza do not represent a single group, and a variety of fungi, including Phycomycetes, are represented.

In 1904 Hiltner described how the surface of roots was colonized by bacteria and he coined the term rhizosphere for the soil volume immediately influenced by the roots. Since Starkey (1929) reviewed what was then known of the influence of higher plants on soil micro-organisms, the microflora of the rhizosphere has been the subject of much investigation. The rhizosphere has been shown to be a zone of stimulation of many organisms, particularly certain groups of bacteria, the stimulation being due to release of nutrients by the plant as root exudates or as moribund root cells. Many fungi, including saprophytes, plant parasites and mycorrhizal fungi, occur on root surfaces and in the rhizosphere (Garrett, 1956).

Much of the work on fungal floras of the soil has been essentially floristic, but more recently there has been emphasis on the ecology of fungi in soil, on the habitats of individual species and the parts they play in the biochemical processes that take place in soil. The general picture of fungi in soil which seems to be emerging from many recent studies is one of organisms consisting mainly of resting structures in a mosaic of micro-habitats, often making little mycelial growth but bursting into activity when some event brings fresh nutrient to resting cells able to exploit it. Root growth, litter accumulation and the activity of the soil fauna play important parts in producing such events. Much detail is, however, lacking. Further, so little is known about some fungi in soil that we cannot be sure that we are not looking at merely half of the picture.

II. METHODS OF STUDY

The methods that have been used to study fungi in soil are considered in detail for, as with any branch of science, it is a truism to say that knowledge is governed by the techniques available and progress is intimately linked with the development of new approaches and methods. Thus evaluation of present knowledge on fungi in soil is largely dependent on understanding the techniques by which the information has been obtained.

Compared with some other fungal habitats, soil has proved difficult to study. This is a consequence of the multitude of organisms that occur in soil, of the complexities of fungal life cycles, together with the difficulties inherent in investigating soil because of its opacity, its heterogeneous nature and its complex structure.

Fungi may occur in soil as mycelium, as fructifications or as a variety of inactive spores. As has recently been re-emphasized (Garrett, 1955; Harley and Waid, 1955) one of the first requirements for an ecological study of soil organisms is that the methods must distinguish between organisms which are vegetatively active and playing a part in the soil processes and those which exist in a dormant or inactive form as spores and other propagules. Many of the methods that have been used, in fact, do not give this information (Warcup, 1960). In attempts to overcome these problems, a wide range of methods, both microscopic and cultural, have been used to study fungi in soil (Durbin, 1961).

In general there have been two different approaches to the study of fungi in soil. The first is by microscopic examination either of soil or of substrates or materials, such as glass or nylon, after they have been placed in soil; the second is by isolation of organisms, either directly or by cultural techniques. Each approach has both advantages and disadvantages.

A. Direct Observation Methods

Kubiena (1938) approached the problem directly by observing fungi actually growing in the soil, using a microscope equipped with a normal incidence illuminator. His observations were necessarily confined to naturally or artificially exposed soil surfaces but yielded information not previously obtained by other methods, While other investigators have used direct observation (Chesters, 1948) this approach has been rather neglected. Surfaces need not always be examined in the field, but freshly exposed surfaces of soil blocks may be examined in the laboratory (Warcup, 1957).

1. Soil sections

Several workers have prepared sections of soil. Kubiena (1938) used a thermolabile plastic material to prepare sections of soil, but his method has not been followed extensively. Haarløv and Weis-Fogh (1953, 1955) impregnated soil with agar for sectioning. An undisturbed sample of soil was soaked in a hot, 2% aqueous solution of agar, cooled, hardened in alcohol and sectioned as thinly as the largest mineral particles would allow. From sandy soils good serial sections of 750 μ thickness were obtained while organic soils were cut at 100 μ. Alexander and Jackson (1955) adapted standard geological techniques for sectioning rock to prepare sections of soils. The method involves impregnation with a synthetic resin and final preparation of sections by cutting, grinding and polishing. Sections about 100 μ thick can easily be obtained. Hepple and Burges (1956) and Burges and Nicholas (1961) used a similar method but with different resins, and obtained sections 50–60 μ in thickness. Minderman (1956) froze soil to $-10°$C and infiltrated it with gelatin which was then fixed in formalin. The sample was then treated with hydrofluoric acid to dissolve sand grains before preparing sections. Sections as thin as 7·5–10 μ were obtained.

These methods are of use in studying micro-organisms in their natural relationships to soil structure, although the methods which involve desiccation before embedding have been criticized (Harrløv and Weis-Fogh, 1955) because desiccation changes the texture of those soil layers in which most of the organic activity is concentrated.

2. Soil staining

Conn (1918) seems to have been the first to stain soil suspensions. He prepared an infusion of soil (1:9) in dilute gelatin (0·015%) and spread 0·1 ml of this across a slide, staining it with rose bengal; erythrosin (Cholodny, 1930) may be used instead. A more recent staining procedure is that of Jones and Mollison (1948), who suspended soil in melted and cooled 1·5% agar. A drop of the agar suspension is placed on a haemocytometer and a coverslip quickly added. The film obtained is floated off on sterile water, placed on a microscope slide, allowed to dry, then stained with phenolic aniline blue and made into a permanent mount. Hyphae were measured as total length per g of soil.

Staining methods allow organisms to be seen and counted, but their relation to soil structure is, in general, lost. It should perhaps be noted that the Jones and Mollison technique tends to neglect the heavier soil particles with which many organisms are associated.

3. Slide or burial techniques

A different approach, but still predominantly observational, is that of the burial methods such as Rossi-Cholodny slides. Rossi (1928) pressed a clean microscope slide against a freshly exposed soil surface so that soil particles and microbial colonies adhered to the slide. After removal and staining, the soil impression slide depicted micro-organisms as they actually occurred in the soil at that time. He also buried slides in soil for different periods. This latter method was perfected by Cholodny (1930) who first brought it to the attention of most workers and it has become known as the Rossi-Cholodny or contact-slide method. It has become the most widely used *in situ* method.

Demeter and Mossel (1933) used the method to detect changes in the population of a field soil and it was used in the laboratory by Conn (1932), who considered it satisfactory to demonstrate a change in the microflora of soil from fungi or actinomycetes to bacteria. Eaton and King (1934) employed the method to ascertain the time of the year at which growth of *Phymatotrichum omnivorum* occurred. Jensen (1934, 1935) adapted the method for quantitative study by estimating the frequency of fungal hyphae in 500 randomized microscopic fields. Starkey (1938) studied the occurrence of micro-organisms in relation to plant roots by letting the roots grow against buried slides. Blair (1945) used the method to study the growth of *Rhizoctonia solani* through soil in the laboratory.

It should be noted that there is an essential difference between soil impression slides and Rossi-Cholodny slides. The former indicate fungal occurrence

at the time of examining a soil, the latter provide a substrate for fungal growth after the soil has been disturbed. There is strong circumstantial evidence that fungal growth on buried slides may be influenced by the disturbance of the soil in burying the slides. Sewell (1959b), noting the frequency of species of *Mortierella* on Rossi-Cholodny slides, remarks that "either by reduction of the soil fungistatic factor or by effecting changes in local soil conditions, or both, the immersion in soil of solid inert objects might produce a physical rhizosphere' within which certain fungi normally quiescent or sparsely growing are stimulated to vigorous growth and consequently are isolated so frequently by direct methods as to misrepresent their real occurrence." Brown (1958a) used impression slides similar to those of Rossi, but smeared the slides with nitro-cellulose thinned to a suitable consistency with amyl acetate to aid retention of soil on the slide.

Instead of glass slides, Waid and Woodman (1957) buried nylon mesh in soil. After periods of burial up to several months the gauze was removed and fungal activity estimated by counting the number of hyphae per mesh.

4. Observation boxes

Another observational method is the use of an observation box (Dean, 1929; Linford, 1942; Sewell, 1959c; Parkinson, 1957). Slides, coverslips, etc., can be incorporated into the side of a box containing soil in which plants may be growing, permitting microscopic examination at a high magnification under reflected light.

While microscopic methods give information on the location and form of fungi in soil, all direct observation methods suffer from the fact that the majority of the mycelia seen in soil or on slides are without fructifications and hence cannot be identified. Since the number of different fungi found in any soil is large, this is a serious handicap and it is probable that the tedium of examining slides, together with the difficulty of identifying the fungi present, have discouraged the use of direct observation methods.

B. Isolation Methods

Most workers who have studied fungi in soil have used isolation methods because these, in general, allow identification of the organisms obtained. It seems probable, also, that well-prepared isolation plates, such as soil dilution plates, have direct aesthetic appeal. Most isolation techniques, however, are indirect methods and it is difficult to tell whether the fungi growing on the plates arise from active mycelia or from inactive spores. This affords a marked contrast with direct observation methods, a contrast that has been epitomized by Garrett (1952): "with the plate count one identifies what one cannot see (i.e. *in situ*), whereas with the direct method one sees what one cannot identify." Recognition of these difficulties has led to much work on isolation techniques in relation to the study of active mycelia in soil. While isolation methods have recently been discussed (Warcup, 1960), they are treated in

some detail here since understanding of the type of information obtained by different methods is essential for an understanding of soil mycology.

1. The soil dilution plate method

The classical and most widely used isolation method is the soil dilution plate method (Waksman, 1927; Garrett, 1951; Warcup, 1960). The method consists of shaking a known amount of soil in sterile water, then obtaining a progressive series of dilutions. From one or more of the dilutions, 1 ml samples are placed in Petri dishes and dispersed with melted but cooled agar. The effect of these various operations on the degree of variability in estimated numbers has been studied extensively (Brierley, Jewson and Brierley, 1927; Bisby, James and Timonin, 1933; James and Sutherland, 1939; Waksman, 1944; Montégut, 1960).

Since there are normally more bacteria than fungi in soil it is necessary to suppress them on isolation plates. To reduce the growth of bacteria and Actinomycetes on soil dilution plates, Waksman (1922) and Jensen (1931) adjusted the medium with sulphuric acid to about pH 4·0; other acids, lactic, boric, and phosphoric, have also been used. Acid, however, is known to depress or prevent the growth of some fungi (Thornton, 1956a). Smith and Dawson (1944) proposed the use of rose bengal at a concentration of 1:15,000 as a bacteriostatic agent, which Dawson and Dawson (1946) found to produce no fungistatic effect other than a reduction of colony size; Martin (1950) recommended the use of a peptone-dextrose agar containing 1:30,000 rose bengal and 30 μg/ml streptomycin or 2 μg/ml aureomycin (chlortetracycline). Pugh (1958) and Warcup (1960) recorded, however, that rose bengal inhibited the growth of some mycelia. Pady, Kramer and Pathak (1960) noted suppression of fungi on media containing rose bengal if exposed to bright light. The effect of light in depressing growth or killing fungi on certain media has also been noted by Weinhold and Hendrix (1962), Nash and Snyder (1962) and Kerr (1963). While antibiotics used either singly or in combination (Dulany, Larsen and Stapley, 1955) are more satisfactory than acidification for suppression of bacteria, the growth of some fungi may also be suppressed by antibacterial antibiotics. For instance, Hine (1962) reported that whereas *Pythium aphanidermatum* and *P. ultimum* grew in the presence of 100 ppm of streptomycin, *P. arrhenomanes*, *P. graminicolum* and *P. mamillatum* were inhibited by much lower concentrations; Schmitthenner (1962) found that chloromycetin even at 5 mg/l partially inhibited *Pythium* spp.; streptomycin is also inhibitory to certain isolates of *Phytophthora* (Eckert and Tsao, 1962).

Several chemicals, including sodium deoxycholate, oxgall, sodium propionate, pentachloronitrobenzene (PCNB), or rose bengal, have been used to retard fungal colony growth and thus minimize the degree of interference between developing colonies on isolation plates (Papavizas and Davey, 1959b; 1961a). Paharia and Kommedahl (1956) reported that distributing 1 ml of soil solution over the solidified agar surface 2–3 days after the plates were poured gave more colonies than incorporating the soil dilution at the time of pouring the plates, especially in the presence of streptomycin and rose

bengal. James (1959) found that soil extract agar and Martin's agar with soil extract were superior to Martin's without soil extract. Soil extract, however, is not always superior (Johnson and Manka, 1961); the difference may be due to differences in the soil extracts used by different workers. Miller (1956) showed that potato dextrose agar and "V-8 juice" agar contain copper in amounts sufficient to be toxic to some fungi, particularly Phycomycetes.

By the soil dilution plate method, the number of colonies/g oven-dry soil may be obtained, also species may be isolated for compiling species lists. It was early realized that the "number" of fungi in soil has little meaning since during the manipulations a single hypha may break into fragments each of which would count as one while a single cluster of spores might be counted as thousands. Further, the method has always been considered to be highly selective, particularly for species that spore abundantly, since many fungi known to occur in soil are rarely isolated on soil dilution plates (Brierley, 1923; Chesters, 1949). Direct evidence for this view has recently been obtained (Warcup, 1955b, 1957). Dilution plates after a short incubation were searched for young fungal colonies, each of which was removed in a small block of agar for direct examination. After the nature of the propagule had been determined, the colony was transferred to fresh medium to permit growth and identification. In this way the majority of colonies developing on dilution plates prepared from samples of wheat-field soil were found to have arisen from spores. Comparative studies showed that not only did dilution studies neglect a large number of fungi but that many of these were present in soil as hyphae.

Warcup (1960) concluded that the dilution plate method is of little value in estimating the activity of fungi in soil. This view has been questioned by Griffiths and Siddiqi (1961), who consider that while a single quantitative estimate from dilution plates is of very restricted value, this is not necessarily true of a succession of estimates made at relatively frequent intervals, for here it is possible to detect changes in populations of spores. They suggest that the spore population may be regarded as a barometer of fungal activity and, just as with an ordinary barometer, it is change in value rather than absolute value which is of interest. This viewpoint has merit but it should always be borne in mind that change in spore number may occur without mycelial activity. For instance, decay of roots or fragmentation of debris may increase spore number without fungal growth. Further, while it is undoubtedly correct to hold the view that even if soil contains a large number of inert conidia these must have resulted from previous mycelial activity, yet this is of little help in elucidating the dynamics of soil populations.

While there has been much criticism of the soil dilution plate method, it has been of great value and, if due regard is paid to its known limitations, it is a most useful means of investigating certain aspects of soil mycology.

2. The soil plate method

In this method (Warcup, 1950, 1960) a small quantity of soil is dispersed throughout a thin layer of agar medium in the isolation plate. The method

was devised after it was observed that in the preparation of dilution plates many fungi are discarded with the residue; it also dispenses with the preparation of water blanks. To prepare soil plates from soils with a high number of colonies/g, Johnson and Manka (1961) diluted the soil with sterile sand.

While incorporation of soil particles with agar should allow hyphae present to grow, recent data (Warcup, 1957) suggest that this may not be so. Colonization of the agar is dependent upon fungal growth rate, and usually the number of fast-growing organisms present as spores in a soil is sufficient to mask growth from viable hyphae. In any case, without further evidence, it is impossible to tell which fungi on a soil plate may have developed from hyphae.

In a comparison of soil dilution and soil plates, Warcup (1957) concluded that both methods give essentially the same picture of the fungal flora of the soil, though soil plates tend to favour faster-growing species present in soil in relatively low number. The chief advantage of soil plates is their ease of preparation. Since they incorporate all the soil, soil plates in conjunction with selective media or other selective isolation procedures may be of more use than soil dilution plates. Park (1961a) has used soil plates incubated in an atmosphere of CO_2 to isolate *Fusarium oxysporum* from populations of less than 10 units/g soil.

3. The direct inoculation and the soil desiccation methods

These methods, more of historical than of practical significance, were both devised to try to answer the question of what fungi are present in soil as mycelia. In the direct inoculation method, Waksman (1916a) transferred lumps of soil, about 1 cm in diameter, on to sterile plates of Czapek's solution agar. After incubation at 22° C for 24 hours, hyphal tips were removed. Waksman remarked: "the organisms thus isolated are believed to come from the mycelium that is actually found in the soil. The period allowed for incubation was not long enough for spores to germinate and produce such a mass of mycelium." Saitô (1955a) and Warcup (1960) found, however, that while Mucorales were isolated from soil lumps, direct microscopic examination of lumps failed to reveal phycomycetous hyphae, thus suggesting that the mycelia developing were derived from spores.

McLennan (1928) suggested that a possible method for discriminating between fungal mycelium and spores in soil might be drying the soil over calcium chloride in a desiccator. Her experiments showed that mycelium was killed by this treatment whereas spores were not. Eastwood (1952) with fungi in pure culture and Warcup (1960) with natural soil found that besides its effect on mycelium, desiccation over calcium chloride caused a marked loss of viability of spores.

4. The immersion techniques

Several general techniques have been devised in which a substrate for fungal growth is placed in soil.

The earliest is the Rossi-Cholodny slide technique; a few workers (Chesters

and Thornton, 1956) have attempted isolation from fungal hyphae growing on such slides. Gams (1959) used nylon strips and isolated fungi by placing the strips on agar media. Tribe (1957a, 1960a, 1961) buried pieces of Cellophane attached to coverslips in soil. After different periods of time and at different stages of decomposition of the Cellophane the coverslips were removed and examined microscopically. Fungi may also be isolated, care being taken that growth occurs from hyphae in the Cellophane and not from attached soil particles. Lens tissue paper has been used in a similar way (Griffiths and Jones, 1963).

Immersion tubes (Chesters, 1940, 1948) and screened immersion plates (Thornton, 1952, 1956a, 1958) resemble each other in that both attempt the isolation of mycelium from soil and both introduce an agar medium into natural soil. Immersion tubes consist of glass tubes, with 4–6 spirally arranged invaginated capillaries, filled with a nutrient agar. A soil core is removed in the field and an immersion tube inserted in its place. After 7–14 days it is removed, and fungi isolated from it by removing a core the length of the tube which is then cut into portions which are plated out. Mueller and Durrell (1957) and MacWithey (1957) have described modifications of Chesters' immersion tubes. Screened immersion plates consist of a distilled water agar-coated glass slide carried in a "Perspex" box with a lid containing 10 spaced holes for entry of fungi. After a suitable period of burial in soil, the plates are removed, examined and fungal growth transferred to potato-dextrose agar. Further immersion techniques have been reported by La Touche (1948), Sewell (1956), Wood and Wilcoxson (1960) and Andersen and Huber (1962).

Chesters and Thornton (1956) compared the fungi isolated from a forest soil by immersion tubes and by screened immersion plates. Discussing the results, they remark that colonization of immersion tubes depends on the ability of individuals to compete successfully with other members of the soil population for entry through capillary orifices (Chesters, 1948; Nicot and Chevaugeon, 1949). Also, once established, the fungi must be capable of growing into the depths of the medium far enough to be isolated in the agar core. Sewell (1959b) noted that fast-growing fungi once established may colonize the bulk of the agar in the tube, and therefore be recorded with a high frequency of isolation. Brown (1958b) recorded that *Trichoderma viride*, by its vigorous growth on the agar film, often excluded the entry of other species into Sewell soil traps.

While it is considered that immersion techniques isolate active mycelium from soil, and the isolation of *Rhizoctonia* and *Armillaria* (Chesters, 1948) and *Rhizoctonia*, *Papulaspora* and other non-sporing mycelia (Thornton, 1956b, 1960) substantiate this, it must be pointed out that there is an element of doubt about sporing fungi isolated, since Dobbs and Hinson (1953, 1960) found that spores may germinate in water that condenses on glass surfaces buried in soil. There is also the possibility that mites and other small soil animals may carry spores on to the buried agar (Warcup, 1960; Dobbs and Hinson, 1960).

5. Soil partition methods

Several methods have been devised for fractionating or partitioning soil, usually into particles of various sizes (Chesters, 1948; Parkinson and Williams, 1961), or sometimes of a particular type (Warcup, 1955a; Levisohn, 1955; Ohms, 1957; Boosalis and Scharen, 1959). Most methods have used sieves of varying sizes, but sedimentation, centrifuging and impaction have also been used; the aim of most techniques has been the removal of fungal spores and fine soil particles and the examination of other units in soil.

Warcup (1955a) reported a simple method for isolating hyphae from soil; essentially the method depends on the fact that when a soil suspension is prepared, many of the fungal hyphae remain with the heavier soil particles of the residue. Removal of the fine suspended matter from the residue also permits visual examination of the latter for hyphae, which may then be removed and grown on agar media. Soils high in organic matter may be more difficult to examine than soils with a high proportion of fine particles. The location of and examination of hyphae on the isolation plates, though tedious, is essential since hyphae may have small humus particles or occasionally spores along their surfaces and growth may originate from these attached particles. However, with suitable precautions one may be sure that growth is from a hypha and no other fungal unit.

In a study of the fungal flora of a wheat-field by dilution plates and by hyphal isolation, Warcup (1957) found that a high proportion of the fungi obtained by hyphal isolation were rare or absent from dilution plates. In contrast to dilution plates, where the most abundant fungi obtained were species of *Penicillium*, *Rhizopus*, *Mucor*, *Cladosporium*, and *Fusarium*, hyphal isolation gave many fungi which remained sterile in culture. Some have since been found to be Discomycetes or Basidiomycetes. While hyphal isolation records many of the fungi present as mycelium in soil, it gives no information on fungi present as mycelium on, or in, residues, large fragments of organic matter, or roots.

Sclerotia may also be obtained from soil by examination of the heavier soil particles following sedimentation or sieving (Warcup, 1959).

Following sieving and comminution of plant debris obtained from soil, Boosalis and Scharen (1959) found by microscopic examination that *Aphanomyces euteiches* may overwinter as oospores embedded in dead plant tissue. They also isolated *Rhizoctonia solani* by plating out similarly treated debris. Levisohn (1955) isolated *Boletus* from soil by picking out rhizomorphs, surface sterilizing them in 0·1% HCl, then plating them on agar media; surface sterilization is not always necessary (Warcup, 1959).

Ohms (1957) obtained large numbers of vesicles of a phycomycetous endophyte by sieving soil and then separating the vesicles from accompanying particles by centrifuging in tubes containing sugar-water mixtures of different densities. Ledingham and Chinn (1955) used a flotation method to recover spores of *Helminthosporium sativum* from soil. Soil mixed with a small amount of mineral oil is shaken up with water; the emulsion which collects on the surface of the water contains most (80–90%) of the spores of *H. sativum*.

The method is suitable also for other fungi with spores with hydrophobic surfaces. Parkinson and Williams (1961) have used a series of washing boxes to fractionate soil into particles of various sizes.

A radically different method for obtaining soil particles of particular size is through use of the Andersen sampler (Buxton and Kendrick, 1963). Weighed amounts of lightly pulverized soil were drawn through a perforated aluminium disk and impacted on agar media, the soil being dispersed uniformly into 400 equal-sized units per dish. Buxton and Kendrick found that this method gave more reproducible counts of *Pythium* and *Fusarium* than did soil dilution plates.

6. Isolation from roots and debris

Since roots and larger pieces of debris constitute major substrates for fungi in soil, any investigation into fungal occurrence and activity in soil needs to consider the growth of fungi on and in these substrates. Roots have long been examined for fungi, particularly for root-disease organisms, mycorrhizal fungi or in connection with rhizosphere studies; larger pieces of debris in soil can be examined in a similar way. Rhizosphere studies have mainly been made by dilution techniques so that little information on activity of fungi is obtainable from them (Harley and Waid, 1955). Pathological investigations have often been made by plating out suitable pieces from the edges of lesions following some sterilization procedure. In some cases, and particularly with fine roots, surface sterilization may kill all organisms in the root, so washing techniques have been used (Simmonds, 1930).

Washing techniques have been used by many workers for the study of fungi particularly on root surfaces (Kurbis, 1937; Simmonds and Ledingham, 1937; Robertson, 1954; Stenton, 1958; Parkinson and Kendrick, 1960). Harley and Waid (1955), in a study of the fungi on roots and decaying petioles in soil, gave the material serial washings in sterile water and by plating out portions of the wash water checked how effectively fungal units were removed. They noted that populations growing on agar from unwashed surfaces are different from those obtained from washed surfaces, because in the former sporing Hyphomycetes are greatly over-estimated, whilst Phycomycetes and more particularly slow-growing non-sporing mycelia are under-represented. The difficulties in removing fungal spores from roots and other surfaces in soil have often not been fully appreciated (Stenton, 1958).

A further difficulty in isolating from roots or other larger fragments is that on plating out such material on agar usually only the faster-growing species survive, overgrowing any other fungi. Stover (1953b) macerated banana roots in a Waring Blender, and reported a greater range of fungi by this technique than from plating root segments. For examining small individual roots, Warcup (1959, 1960) used a root-fragmentation method and isolated many slower-growing non-sporing fungi, including Basidiomycetes, from the roots of pasture plants. Fragmentation of some roots, however, may reduce the number of colonies obtained, owing to release of toxic materials (Clarke and Parkinson, 1960).

With some fungi, including plant pathogens, it may be more advantageous to keep roots or debris in a moist chamber than to plate out on agar (Taubenhaus and Ezekiel, 1930; Keyworth, 1951; Butler, 1953).

Wilhelm (1956) used a different approach for examining fungi occurring on roots. He surface-sterilized roots, often whole root systems, with mercuric chloride, and buried them for 2–4 weeks in sterilized moist sand. Roots were then examined for resting or reproductive structures of various fungi; these were isolated for further study.

7. *Selective methods*

A range of selective methods has been used to isolate fungi from soil. Selective methods are especially valuable when the organisms are present in soil in low numbers.

A method especially profitable with species of *Pythium* and other water-moulds is to isolate from hemp seed floating in water covering the soil sample. Papavizas and Davey (1959a) have used stem pieces of mature Buckwheat (*Fagopyrum esculentum*) for isolation of *Rhizoctonia solani* from soil; Baker (1953) used rose stick baits for *Chalaropsis thielavioides*. Many workers have used straw burial techniques such as that devised by Sadasivan (1939) to isolate fungi from soil. Yarwood (1946) isolated *Thielaviopsis basicola* with carrot disks; to prevent bacterial rot, soaking the disks in 0·05% streptomycin has been recommended (Maloy and Alexander, 1958). Lloyd and Lockwood (1962) have cautioned, however, that carrots bought in plastic bags in the U.S.A. may themselves be contaminated with *Chalaropsis thielavioides* and *Thielaviopsis*. Campbell (1949) inoculated apples with soil for isolation of *Phytophthora cinnamomi*; Newhook (1959) found that better results were obtained if the soil to be tested was soaked for two days before being inserted into the apples. Pineapple leaves have also been used for *P. cinnamomi* (Anderson, 1951) and lemons for *P. parasitica* and *P. citrophthora* (Klotz and Fawcett, 1939). Cellophane, boiled grass leaves, pine pollen, shrimp chitin, insect wings, hair, and cast snakeskin have been used as bait for soil chytrids (Sparrow, 1957); hair has also been used for dermatophytes (Vanbreuseghem, 1952; Griffin, 1960) and other fungi. Dermatophytes have also been isolated by the mouse-injection method (Emmons, 1951). Many workers have used hosts as selective media for isolation of soil-borne pathogens; hosts are often the only means of showing the presence in soil of pathogens of aerial parts of plants. Maloy and Alexander (1958) have also used hosts as selective media for a "most probable number" method for the estimation of populations of plant pathogens in soil.

8. *Selective media*

In general, mycologists have had less success than bacteriologists in their search for selective media, though they have well realized how much easier isolation would be by use of selective media. Recently, however, with the spate of antibiotics and antifungal agents being produced commercially, the

possibilities of obtaining good selective media have become much more promising. Vaartaja (1960) screened a range of fungicidal materials in agar culture and found that many had a selective action on 10 species of fungi representing different fungal groups. He considered that this selectivity might be utilized in isolating fungi; for instance the polyene group of antibiotics are tolerated by *Pythium* and *Phytophthora* but not by most other organisms; limited tests suggested that *Phytophthora cactorum* can be separated from the usually faster-growing *Pythium* through its better tolerance of tannins; compound B22555 (Dexon) was highly specific against Pythiaceae.

Eckert and Tsao (1962) have used pimaricin for isolating *Phytophthora* and *Pythium* from root tissues; on media incorporating pimaricin no fungi other than *Pythium* and *Phytophthora* were obtained. Singh and Mitchell (1961) used both PCNB and pimaricin for making a selective enumeration of *Pythium* in soil platings. Since pimaricin rapidly deteriorates at high temperatures and in the presence of moisture and sunlight, it should be used immediately after the suspension is prepared. Schmitthenner (1962) has used endomycin for Pythiaceae because it is more stable than pimaricin. Singh and Mitchell also stress the need for caution in selecting media to be used with inhibitors since PCNB and pimaricin adversely affected *Pythium* when used in media containing oxgall.

Russell (1956) reported a medium using *o*-phenyl phenol for selective isolation of Basidiomycetes from wood pulp and from air in pulp mills; in my experience this medium is not successful for isolating Basidiomycetes from soil. Parmeter and Hood (1961) have used autoclaved culture filtrates of *Fusarium solani* f. *phaseoli* incorporated in agar as a medium to isolate that strain from soil. Isolates developed their characteristic pigment and sporulated well but competing fungi, including *F. oxysporum*, were restricted on the culture-filtrate medium. Martinson and Baker (1962) found that exudates from radish combined with media significantly increased the isolation of *Rhizoctonia solani*.

If good selective media become available, it may be necessary to reconsider present isolation procedures; it may no longer be necessary to fragment tissues to isolate slow-growing fungi; it may be simple to demonstrate a low number of units of a fungus in soil. Caution, however, will be necessary to make sure that no form (mycelium, resting structures, or spores) of a fungus is inhibited by the selective substances used.

III. THE FUNGI OCCURRING IN SOIL

Although floristic lists of soil fungi have been compiled since the end of the last century, it is of interest that we are still unable to give an adequate picture of the fungal flora of a soil. This is because most of our knowledge of the fungi present in soil has been derived from studies using the soil dilution plate method, and because many fungi do not sporulate readily on agar media.

A wide range of soils under many different types of vegetation and from

many different geographical areas has been examined for fungi. A partial list of such investigations includes: fungi from coniferous forest soils (Morrow, 1932; Ellis, 1940; Kendrick, 1958), deciduous forest soils (Tresner, Backus and Curtis, 1954; Krzemieniewska and Badura, 1954; Witkamp, 1960; Christensen, Whittingham and Novak, 1962), heath soils (McLennan and Ducker, 1954; Sewell, 1959d), peat soils (Stenton, 1953; Moore, 1954), grassland soils (Warcup, 1951a; Orpurt and Curtis, 1957; Apinis, 1958), recently glaciated soils (Cooke and Lawrence, 1959), desert soils (Nicot, 1960; Durrell and Shields, 1960), tropical soils (Farrow, 1954), mangrove swamps (Swart, 1958), sand dunes (Webley, Eastwood and Gimingham, 1952; Saitô, 1955b; Brown, 1958b), salt marshes and mudflats (Bayliss Elliot, 1930; Saitô, 1952; Pugh, 1960), and cultivated soils (Nicot, 1953; Miller, Giddens and Foster, 1957; Guillemat and Montégut, 1957; Warcup, 1957; Joffe, 1963).

Early studies by the soil dilution plate method in various countries showed that certain genera of fungi were commonly found in soil; this observation led Waksman (1916b, 1917) to postulate that there is a cosmopolitan fungal flora of the soil. Waksman studied the fungi occurring in 25 soils from North America and Hawaii and collated his results with those obtained by investigators in different parts of Europe. Since *Aspergillus*, *Mucor*, *Penicillium* and *Trichoderma* were found in all investigations, Waksman concluded that these organisms, associated with several of the following, *Zygorrhynchus*, *Cladosporium*, *Alternaria*, *Rhizopus*, *Fusarium*, *Verticillium*, *Cephalosporium*, *Acrostalagmus*, *Scopulariopsis*, *Botrytis*, some sterile mycelium, and some yeasts, made up the fungal flora of a soil. Species of these genera are still prominent in lists of soil fungi today (Gilman, 1957). Burges (1958) has pointed out that this apparent uniformity of the soil flora may be largely an artifact, based partly on uniformity in the method of isolation, and partly on the fact that the genera quoted all have fairly characteristic sporing structures that allow easy identification at least to genus, whereas isolates which are difficult to identify are seldom included in lists of soil fungi. It also ignores what is known of the distribution of fructifications of the larger Ascomycetes and Basidiomycetes. Populations of these fungi may be different for a forest, a meadow or a heath; one of the most obvious specializations in habitat is that of coniferous forests in contrast with dicotyledonous (Corner, 1950).

The soil dilution plate method has been used to obtain the "number" of fungi in soil and also to study the diversity of the soil flora. It was early realized that many fungi known to fruit on soil did not occur on soil dilution plates. Brierley (1923) remarked "that of the multitudes of Basidiomycetes growing in wood and meadow not one should have been recorded is indeed startling. It was at first thought that many imperfect fungi might be conidial stages of Basidiomycetes, but much search at Rothamsted has, up to the present, failed to reveal clamp connections in the hyphae." Bisby, Timonin and James (1935) point out that there are records of some 670 species of Basidiomycetes in Manitoba, the large majority of which grow "on the ground," yet these fungi were virtually absent from their isolations from soil.

While it is known that most larger Ascomycetes and Basidiomycetes do not sporulate readily, if at all, on agar media, dilution studies usually fail to reveal non-sporing mycelia which could represent these fungi. Chesters (1949) emphasized that while a great deal of labour has been expended on the estimation of numbers and species of fungi in soil it must be understood that these labours have been successful only in part. He considered, that, quite apart from the higher Basidiomycetes, such frequent inhabitants of the soil as species of *Pythium*, species of *Mortierella* and a galaxy of the darker Hyphomycetes are seldom, if ever, reflected in their true relationships in dilution plate studies.

The species recorded by Gilman (1957) provide an interesting commentary on present knowledge of the fungi isolated from soil. Gilman lists over 690 fungi in 170 genera as having been isolated from soil; however, 10 genera, *Penicillium*, *Fusarium*, *Mucor*, *Aspergillus*, *Achyla*, *Mortierella*, *Pythium*, *Chaetomium*, *Saprolegnia* and *Monosporium*, account for more than half the species isolated. Gilman lists 80 Ascomycetes, 2 Mycelia Sterilia, and no Basidiomycetes. Burges (1958) commented that, while it would be unjustifiable to place great importance on any particular one of these figures, nevertheless they reflect quite fairly the general picture of soil fungi as determined by the dilution plate method. With the reservation that Gilman's book is a compilation, this is probably so apart from the species of watermoulds, *Achlya*, *Saprolegnia*, etc., most of which were not isolated by the soil dilution plate method. Some workers are of the opinion that soil mycologists must accept such genera as *Mortierella*, *Penicillium* and *Aspergillus* as dominant and active members of the soil population; most workers, however, find it difficult to reconcile the lists of fungi isolated by the dilution plate method with many of their general observations of fungal activity in soil, and also with the diverse range of organisms obtained by selective techniques.

Robertson (1954) and Harley and Waid (1955) showed that on many living mycorrhizal root systems cleansed of fungal spores by prolonged washing, there occurred many non-sporing mycelia which were not obtained when unwashed root surfaces or dilutions of washings from root surfaces were cultured. Similar mycelial forms had been encountered by many earlier workers on mycorrhiza. Since such non-sporing mycelia were not known from soil or litter fragments, Harley and Waid suggested that they might perhaps belong to the group of "root-inhabiting" fungi which had been distinguished from the actively saprophytic and rapidly-growing "soil-inhabiting" fungi by Garrett (1951).

Warcup (1955a, 1957) investigated the occurrence and activity of fungi in a wheat-field soil by plating techniques and by direct isolation. He found that a high proportion of the fungi obtained by hyphal isolation were non-sporing species which were rare or absent from soil dilution or soil plates. *Rhizoctonia solani*, *Rhizoctonia* spp. and 9 Basidiomycetes were among the 68 non-sporing fungi obtained by hyphal isolation. Thornton (1956b, 1960) found that *Rhizoctonia* occurred in natural grassland soils and formed a high proportion of his isolates on screened immersion plates. Papavizas and Davey

(1962) also isolated many clones of *R. solani* existing saprophytically in soil. Sewell (1959b) and Parkinson and Kendrick (1960) have noted many dark non-sporing mycelia in soil and litter; Saitô (1956), Warcup (1959) and Witkamp (1960) have provided evidence that Basidiomycetes are active in certain horizons of undisturbed and cultivated soils. Listing the fungi isolated from soils in Israel, Rayss and Borut (1958) comment that "many forms of sterile mycelia belonging to the genera *Rhizoctonia, Sclerotium* and others have been isolated by us from different soils without being definitely identified." Evidence is strong that non-sporing fungi have been obtained more frequently by plating techniques than published lists would suggest, but are not included in such lists because they are difficult to identify and because they are found only occasionally and are not usually among the "abundant" fungi. It is also interesting to recall that the fungi seen on Rossi-Cholodny slides are usually sterile. While it is often considered that this may be because conditions favourable for growth need not be suitable for sporulation, it may well be that many of these mycelia, if isolated, would prove to be non-sporing on agar media. The data show that non-sporing fungi are common though not necessarily present in high "number," that they may be isolated from soil, from root surfaces and other microhabitats. Some have clamp connections and may be placed as Basidiomycetes, but the identity of the majority is not known. Another neglected aspect is that both non-sporing *and conidial* fungi may represent imperfect states of Ascomycetes.

Investigation into the identity of non-sporing mycelia obtained from Urrbrae loam at the Waite Institute, Adelaide, is being undertaken, and some progress has been made, particularly with Basidiomycetes and Discomycetes (Warcup and Talbot, 1962, 1963). While the identity of many of the fungi isolated from this soil is not yet known, it may be of interest to list the genera (Table I) which have been obtained from *one* soil. All were obtained from any of two adjacent wheatfields and a sown permanent pasture, all on the same soil type. The fungi were investigated by direct observation, by isolation of hyphae, rhizomorphs, sclerotia or fructifications from soil, by examination of living and dead roots, decomposing residues and other substrates, besides soil dilution plates and soil plates; no special baiting techniques were used.

While a wide range of genera has been obtained, the number of sterile mycelia as yet unidentified is perhaps surprising (Warcup and Talbot, 1962). It should be remembered, however, that not all mycelial isolates may represent different species of fungi. Mycelial isolates are grouped on cultural and hyphal characters into "types." Since it is easier to combine records if two cultural types are found to represent different strains of the same fungus than to disentangle records where more than one fungus is accidently placed under one type, cultures showing some differences are usually kept as distinct types. Sometimes when fructifications are subsequently obtained, different types are found to be the same species; nevertheless, experience suggests that the majority of the mycelial types represent different species of fungi. On the other hand some cases are known where fungi differing widely taxonomically have indistinguishable mycelia. It is noteworthy that of 29 identified

TABLE I

List of genera of fungi obtained from Urrbrae loam

Phycomycetes	
†Chytridiales	‡*Rhizophydium*; ‡polycentric chytrids, 2 spp.
Saprolegniales	‡*Aphanomyces*; *Thraustotheca*
Peronosporales	*Pythium*, 4 spp.
Mucorales	*Absidia*, 3 spp; *Actinomucor*; *Circinella*, 2 spp.; *Coemansia*; *Cunninghamella*; *Gongronella*; *Helicostylum*; *Mortierella*, 5 spp.; *Mucor*, 3 spp.; *Piptocephalis*; *Rhizopus*, 2 spp.; *Syncephalis*, 2 spp.
Entomophthorales	*Conidiobolus*; *Entomophthora*
Ascomycetes	
Eurotiales	*Anixiopsis*; *Arachniotus*; *Aspergillus*, 2 spp.; *Auxarthron*; *Emericellopsis*; *Pseudoarachniotus*; *Spiromastix*
Chaetomiales	*Chaetomium*, 3 spp.
Helotiales	‡*Sclerotinia*
Pezizales	‡*Humarina*; ‡*Anthracobia*; ‡*Ascophanus*
Pleosporales	‡*Ophiobolus*; *Pleospora*
Xylariales	‡Xylariaceae, 2 spp.
Basidiomycetes	
Tremellales	‡*Sebacina*, 2 spp.
Agaricales	‡*Agrocybe*; ‡*Coprinus*; ‡*Leptoglossum*; ‡*Leucocoprinus*; ‡*Marasmiellus*; ‡*Marasmius*; ‡*Omphalina*
Polyporales	‡*Athelia*; ‡*Coniophora*; ‡*Coriolus*; ‡*Corticium*; ‡*Cristella*, 2 spp.; ‡*Hyphodontia*; ‡*Oliveonia*; ‡*Peniophora*, 3 spp.; *Pistillaria*; ‡*Physalacria*; ‡*Sistotrema*, 2 spp.; ‡*Thanatephorus*; ‡*Tomentella*; ‡*Waitea*
Nidulariales	‡*Cyathus*; ‡*Sphaerobolus*
Fungi Imperfecti	
Sphaeropsidales	*Ascochyta*; *Dinemasporium*; *Haplosporella*; *Phoma*, 7 spp.
Moniliales	*Acremoniella*; *Acremonium*; *Alternaria*, 3 spp.; *Aspergillus*, 7 spp.; *Beauveria*; *Botryotrichum*; *Botrytis*; *Brachysporiella*; *Cephalosporium*; *Cladosporium*; ‡*Costantinella*; *Curvularia*; *Cylindrocarpon*, 2 spp.; *Dactylella*, 2 spp.; *Dendryphion*; *Eladia*; *Epicoccum*; *Fusarium*, 4 spp.; *Geotrichum*; *Gliocladium*, 2 spp.; *Gliomastix*; *Gonytrichum*; ‡*Helicodendron*; ‡*Helicomyces*; ‡*Helicosporium*; *Helminthosporium*; *Humicola*; *Metarrhizium*; *Myrothecium*; *Oidiodendron*; *Oospora*; *Paecilomyces*; *Papularia*; *Penicillium*, 16 spp.; ‡*Phialophora*; ‡*Phymatotrichum*; *Pithomyces*; *Podosporiella*; *Pullularia*; *Stachybotrys*; *Stemphylium*; *Stysanus*; *Trichoderma*; *Verticillium*
Melanconiales	*Pestalotia*
Mycelia Sterilia	approx. ‡160 cultures

† Classification after Martin in Ainsworth (1961).
‡ Non-sporing on agar media.

Basidiomycetes 11 lack clamp connections. Besides the fungi listed in Table I, vesicular-arbuscular endophytes are common in roots and in soil, *Olpidium brassicae* has been noted in roots, and a fungus which forms small plate-like sclerotia is common in soil; none of these have been obtained in culture.

Selective methods have shown that other groups of fungi, chytrids (Willoughby, 1961), water-moulds (Harvey, 1925), fungi which attack nematodes, protozoa and amoebae (Drechsler, 1941; Duddington, 1955, 1957), plant pathogens (Garrett, 1956), animal and human pathogens (Ajello, 1956; de Vries, 1962), and mycorrhizal fungi (Harley, 1959), may be isolated from soil or plant roots. Other groups such as *Endogone*, *Tuber*, and the hypogeous Gasteromycetes (Hawker, 1954, 1955a) occur in soil, although, as far as I am aware, they have not yet been isolated apart from their fruit-bodies. Considering also the diversity of habitat of many terrestrial Basidiomycetes and Ascomycetes as shown by occurrence of their fructifications in restricted localities or in association with particular plants, the opinion of Brierley (Brierley, Jewson and Brierley, 1927), that the number and kinds of fungi in soil is legion and that there are perhaps few fungi capable of existing saprophytically which may not sooner or later be cultured from soil, seems very apposite.

How many of the fungi isolated from soil are capable of carrying out their whole life cycle in soil is not known; in fact, life cycles of comparatively few "soil fungi" are known in detail. Burges (1958) for instance, has pointed out that many of the Ascomycetes recorded from soil seem to be more closely connected with dung and animal droppings than with the mineral soil. Besides Ascomycetes such as *Fimetaria* and *Sporormia*, this is probably also true of Phycomycetes such as *Basidiobolus* (Griffin, 1960), *Pilaira*, and members of the Kickxellaceae. While the evidence suggests that these fungi are predominantly dung inhabitants and that they are found in soil as long-lived spores, we do not possess sufficient data to know whether or not they are also capable of growth in certain habitats in soil. It is of interest that some ubiquitous soil fungi such as certain species of *Aspergillus* and *Penicillium* have been found sporing, not in mineral soil, but on the remains of soil animals (Warcup, 1957; Sewell, 1959b).

A. Fungal Structures in Soil

The species of fungi that live in soil are, like other fungi, remarkable for their diversity of form and, being a heterogenous group drawn from different families and orders, vary immensely in size and in the complexity of their life cycles. The vegetative thallus is typically filamentous but may be unicellular. Some species are able to survive throughout the year as mycelium in soil and have no known reproductive structures; others form the complex sporophores of the larger Ascomycetes and Basidiomycetes. Information on the fungal structures that occur in soil has been gained from microscopic examination of soil itself, of soil-contact slides and of washed debris or plant roots. Study of the form of fungi in soil has, however, been a rather neglected field.

1. Hyphae

Most workers have noted fungal hyphae in soil since Frank first noted their abundance in forest litter in 1885. They occur on the surface of mineral particles, traverse soil pores and other spaces and occur on, and in, roots and organic debris. Some hyphae are full of cytoplasm, possess growing tips, stain deeply and are alive and active; others lack contents, do not stain appreciably, are collapsed or shrivelled and are dead. Where hyphae are very fine or possess thick dark-coloured walls, or stain with difficulty, or are of very irregular shape, it may be difficult to decide whether they are alive or dead (Warcup, 1957). Owing to close contact with irregular particles, hyphae in soil, unlike those developing on agar media, are often more or less irregular in shape and size (Saitô, 1955a). Portions of hyphae traversing air spaces may differ in appearance from those parts in close contact with mineral particles. Hyphae in root hairs and plant cells may swell to fill the cavity in which they occur, or become greatly constricted in passing from cell to cell; oidium-like hyphal systems are not uncommon in roots (Hildebrand and Koch, 1936; Nicolson, 1959). Hyphae on particles or on surfaces may be compacted into plates of sheath-like tissue, the extreme of this hyphal form being the hyphal sheaths of ectotrophic fungi on mycorrhizal roots.

Many types of hyphae occur in soil: non-septate phycomycetous hyphae; fine or wide, hyaline or dark-coloured, septate hyphae; hyaline or coloured hyphae with clamp connections. Burges and Nicholas (1961), studying hyphal occurrence in soil sections, divided the hyphae found in a humus-podzol under pine into 6 groups. They consider that with further experience it would be possible to make a more critical division into hyphal groups.

Among phycomycetous hyphae there is considerable range in size and form. Hyphae of *Pythium* and *Mortierella* are often fine, about 2 μ in diameter, but hyphae of some members of the Saprolegniacae such as *Thraustotheca clavata* growing on the surface of buried leaves may be 20–30 μ in diameter. In many Phycomycetes, dense cytoplasm occurs only near hyphal tips; further back the cytoplasm is often vacuolate or the hyphae appear empty.

A type of phycomycetous hypha that is widespread in soil is that of the vesicular-arbuscular endophytes (Peyronel, 1924; Butler, 1939; Gerdemann, 1955; Mosse, 1959; Nicolson, 1959). These are coarse, generally aseptate hyphae with highly characteristic unilateral, angular projections and thick (up to 4 μ), yellowish walls. Such hyphae may carry large terminal vesicles, which are not separated from the hyphae by septa (Butler, 1939; Nicolson, 1959). Besides the thick-walled hyphae there may be a system of thin-walled, often septate hyphae which arise as lateral branches, often from the angular projections of the coarse hyphae (Mosse, 1959; Nicolson, 1959).

Among septate hyphae, a type that is common in soil is the "rhizoctonia-like" hypha (Thornton, 1956b, 1960; Warcup, 1957; Tribe, 1960a). These hyphae are thin to relatively wide (5–20 μ in diameter), and bear characteristically constricted side branches at a wide angle from the parent hypha. A septum occurs near the constriction. Their colour may vary from hyaline to dark brown or black, the coloured hyphae usually possessing thickened

walls. Many fungi with this type of hypha have been classified in the genus *Rhizoctonia*. *R. solani*, the pathogen, with its innumerable strains, is the best-known member of the group but many other Rhizoctonias have been isolated from soil. Some species are known to be Basidiomycetes; however, rhizoctonia-like mycelial states are known for some Ascomycetes such as *Morchella* and *Anthracobia*, and for Hyphomycetes such as *Phymatotrichum* so that this mycelial growth form has wide taxonomic limits. Durbin, Davis and Baker (1955) record that mycelium of *Helminthosporium cactorum* in cacti may be confused with that of *R. solani*, and McKeen (1952) considered that *Phialophora radicicola* may have been confused with *R. solani* because of the branching, septation and brown colour of old hyphae. Examination of septal pores may help to differentiate Basidiomycetes from other fungi with rhizoctonia-like hyphae (Bracker and Butler, 1963).

Many other types of septate hyphae occur in soil but there are comparatively few data on these forms, and even fewer on form in relation to identity. Some Basidiomycete hyphae are notable not only for possession of clamp connections but also for the crystals that encrust them.

It is difficult to decide how many of the hyphae present in a soil are viable. Warcup (1957) found that, on average, 23% of the hyphae washed from a wheat-field soil were viable. This value rose to 75% soon after crop residues were ploughed in and fell to 3–15% during the dry summer. When the soil dried out well below wilting point most hyphae were killed, but a few, including single hyphae besides mycelial strands, survived. In the same soil, but under an old pasture, percentage viability was always lower, ranging from 1–25%. Evidence suggests that in some natural uncultivated soils percentage viability may appear to be even lower. Here, however, even more than in agricultural soil, one has the added complication of hyphae that are viable but do not grow readily on the isolation medium. Other sources of viable mycelia are roots and residues in soil but these habitats are not investigated by the hyphal isolation method. Studies in the old pasture, for instance, showed that although there were comparatively few viable hyphae present in the soil, there was considerable viable mycelium on and in both living and dead roots.

Very little is known of the functional life of an individual hypha in soil, but it is generally considered that hyphae are short-lived through being attacked by other organisms (Waksman, 1927; Starkey, 1938; Russell, 1961; Tribe, 1957a). Sewell (1959b) has commented on the evanescent nature of most hyphae on Rossi-Cholodny slides which had been immersed in a heath soil for only 7 days; it is probable, however, that he was dealing with a restricted part of the mycelial population, since some slower-growing species would not have colonized buried slides in that period. Warcup (1957, 1960) found that some hyphae remained viable in dry soil for over 12 months, but commented that little was known of their ability to survive in moist soil. He found that many hyaline hyphae, both phycomycetous and septate, appeared short-lived in moist soil and soon lost their cell contents when not actively growing, but his data suggested that some dark-brown or black hyphae might be relatively long-lived. Many of the latter may be "resting hyphae." Tracey

(1956) and Witkamp (1960) recorded that dark hyphae appear to persist in soil longer than hyaline hyphae. Other fungi which may have relatively long-lived hyphae in soil include the vesicular-arbuscular endophytes and some Basidiomycetes. Waid and Woodman found that on nylon mesh left in a woodland soil for 410 days a high proportion of the hyphae present were pigmented or had clamps (Waid, 1960). Hyphae in residues appear longer-lived than those on mineral particles or traversing air spaces in soil (Jones and Mollison, 1948; Boosalis and Scharen, 1959).

2. Rhizomorphs

Rhizomorphs or mycelial strands may be common in soil and typically occur when a mycelium is growing over a surface or through a medium having a negligible content of free nutrients. Strands are not usually formed by mycelia growing within substrates. They are formed by some Ascomycetes and Hyphomycetes but are best developed among the Basidiomycetes. Rhizomorphs and mycelial strands are often conspicuous features of the litter and uppermost layers of woodland and forest soils (Mikola, 1956; Thornton *et al.*, 1956; Saitô, 1956; Witkamp, 1960), but also occur in cultivated soils (Warcup, 1959).

Garrett (1951, 1954, 1956) has rejected the earlier explanation that rhizomorphs are primarily a protection against desiccation since, so far as they have been investigated, they are not tolerant of severe drying. For instance the strands of *Phymatotrichum omnivorum* (Texas cotton root-rot fungus) are quickly killed by drying (King *et al.*, 1931). Further, rhizomorphs are common in soils where drought is no problem. Data suggest, however, that the rhizomorphs of different fungi vary in their ability to withstand drying. Many mycelial strands in wheat-field soil at Adelaide remain viable over the summer dry period when soil moisture may be below wilting point for several months (Warcup, 1959). Whether individual hyphae of these strand-forming Basidiomycetes are equally drought-resistant is not known.

3. Vesicles

Although reported by few investigators, vesicles, most of which represent extra-matrical spores of phycomycetous endophytes, are widespread in soil (Gerdemann and Nicolson, 1963). Since vesicles do not germinate on agar media and are present in comparatively low number they are not recorded by plating techniques and other methods must be used to show their presence in soil. They are usually obtained by partition methods, flotation, wet sieving, decanting, etc., thus they have been noted by nematologists (Triffitt, 1935), who commonly use such methods.

Vesicles are spherical, cylindrical or irregularly globose in shape, vary in diameter from 100–800 μ, usually have thick walls, contain many oil globules, and are light yellow to black when mature. Vesicles may occasionally contain other thin-walled, spherical spores; the nature of these internal spores is not known. Recently Gerdemann and Nicolson (1963) have recorded six different

types of vesicles or spores from Scottish soils and have shown that some types produce endophytic mycorrhiza. At least one other type of spore occurs in Urrbrae loam, hence it is probable that others will also be found.

4. Chlamydospores

Chlamydospores are essentially vegetative resting spores with a thickened, often dark-coloured wall. They are typically formed as swollen cells in vegetative hyphae, but may also occur in sporangiophores or in conidia. Chlamydospores of water-moulds are sometimes developed from vegetative cells but are commonly formed from unfertilized oogonia (Hawker, 1957a). In particular species chlamydospores are induced by: a high concentration of sugar (*Mucor*); a reduction in available food (*Saprolegnia*); a low C—N ratio (*Fusarium oxysporum*; Carlile, 1956); by heat treatment (*Fusarium*; Roistacher, Baker and Bald, 1957); by the presence of antagonistic bacteria (*Fusarium*; Venkat Ram, 1952; unknown mycelia; Waid and Woodman, 1957); and in unsterilized soil (*Fusarium*; Nash, Christou and Snyder, 1961; *Mycosphaerella*; Carter and Moller, 1961).

Chlamydospores are generally considered to be formed by relatively few fungi (Hawker, 1957a), but are prominent in many fungi occurring in soil (Park, 1954; Dobbs and Hinson, 1960; Waid, 1960), and are probably formed more commonly than is usually realized. They occur in all major groups of fungi but are best known in the Mortierellaceae, the Mucoraceae, the Saprolegniaceae and the Hyphomycetes, including *Fusarium*, *Cylindrocarpon*, *Trichoderma*, *Botryotrichum* and *Humicola*. They may be formed in the Boletaceae (Pantidou, 1961) and in other Basidiomycetes. Chlamydospores are common in soil but comparatively little is known about them. They may be formed in soil as in *Mucor ramannianus* (Hepple, 1958) or in, or on, plant tissues as in *Fusarium* (Nash *et al.*, 1961; Christou and Snyder, 1962), or *Thielaviopsis* (Christou, 1962a). On decay of the tissue the chlamydospores are released in the soil embedded in small humus particles where, without staining, they are not easily detected by microscopic examination.

5. Sclerotia

Sclerotia consist of closely interwoven hyphae which often lose their original form so that individual cells become globose or tightly compacted. In some sclerotia the outer layer, or layers, may form a thick rind of pseudosclerenchyma often with brown or black walls; others are more or less uniform in structure throughout. Sclerotia vary in size from structures less than 50 μ in diameter (Boosalis and Scharen, 1959) to large sclerotia such as those of the Australian fungus *Polyporus mylittae* which may be 20–30 cm in length (Burges, 1958). *Polyporus tuberaster* in Canada (Vanterpool and Macrae, 1951) and *Poria cocos* in the U.S.A. (Weber, 1929) also form large sclerotia. Sclerotia may germinate directly by formation of hyphae or, as in *Sclerotinia*, *Typhula*, *Polyporus* and *Poria*, give rise to complex sporophores. Sclerotia may be formed in soil itself, on the external parts of plants as with *Rhizoctonia*

solani on potato tubers, or they may occur inside plant tissues as with *Sclerotinia sclerotiorum* on Compositae and Cruciferae and *R. solani* on bean (Christou, 1962b). Much of our information on sclerotia in soil has come from study of soilborne plant parasites many of which, including *Helicobasidium*, *Rhizoctonia*, *Sclerotium*, *Phymatotrichum* and species of the Sclerotiniaceae, form sclerotia (Garrett, 1956).

Reserve foods, including oils and glycogen, are accumulated in sclerotia so that these structures are well adapted for surviving adverse conditions. Many sclerotia can survive long periods in soil. Gadd and Bertus (1928) found *Rhizoctonia* to survive 6 years in dry storage; Ezekiel (1940) reported that 8% of the sclerotia of *Phymatotrichum omnivorum* were still viable after 7 years in moist soil, and Coley-Smith (1959) found that a high proportion of the sclerotia of *Sclerotium cepivorum* (white rot of onions) survived 4 years in undisturbed soil in the field. Garrett (1956), who considers that sclerotia and rhizomorphs are homologous structures, thinks that the protection afforded to sclerotia by thick-walled outer layers does not imply that their primary function is survival of desiccation but that this merely supplements their function of storing food. Hawker (1957b) considers that perhaps both resistance to drought and provision of food reserves are equally important for survival. Drought, however, is not the only significant factor operating against survival of a fungus in soil; protection against other organisms is also important. Ferguson (1953) has shown that nutrient-rich, viable sclerotia are rarely colonized by other soil organisms whereas those killed or injured by various soil treatments are rapidly decomposed. He found that in *Sclerotinia sclerotiorum* the protective mechanism was independent of the thickened rind since both intact and halved sclerotia generally remained free of other organisms.

Studies at Adelaide have shown that some 30 fungi isolated from Urrbrae loam form sclerotia, and a considerable number of these have been isolated from sclerotia from soil in the field. It is interesting that some fungi isolated from sclerotia washed from soil only form indefinite sclerotium-like masses on agar media. Sclerotia have been found in species of *Aspergillus*, *Penicillium*, *Botrytis*, *Cephalosporium*, and *Rhizoctonia* and other non-sporing mycelia, *Leptoglossum*, and in the Basidiomycetes *Coprinus*, *Leucocoprinus*, *Omphalina*, *Cristella*, *Sistotrema* and *Waitea* (Warcup and Talbot, 1962).

6. *Spores*

Fungi produce a wide variety of spores, moreover many species may produce more than one type of spore. Asexual spores (zoospores, non-motile sporangiospores and conidia) are considered to be primarily organs of dispersal (Hawker, 1957b). Their ability to survive unfavourable conditions is limited, but they are often better equipped for survival than is some mycelium. Even naked zoospores of the Saprolegniaceae may rapidly encyst and develop thick walls. Conidia usually have denser cytoplasm than have hyphae and vacuoles are usually absent, factors which aid resistance to unfavourable conditions.

In several groups, as with the oospores of the Oomycetes and the zygospores of the Zygomycetes, the sexual spores are thick-walled resting spores. In Ascomycetes and Basidiomycetes the sexual process results in the formation of characteristic ascospores or basidiospores; these may be resistant spores in some species. Many ascospores, including those of *Byssochlamys*, *Neurospora*, *Aspergillus*, coprophilous Ascomycetes, and the species that fruit on burnt ground, are resistant to heat. Ascospores of truffles and the ecologically similar *Elaphomyces* are so resistant to external changes that they have not been germinated in artificial culture (Hawker, 1954, 1955a). Basidiospores of some Hymenomycetes may survive for several years under dry conditions (Cochrane, 1960), also certain hypogeous Gasteromycetes have resistant spores, but there are comparatively few data for this group.

Many investigators have recorded fungal spores in soil; they have been seen in soil films (Jones and Mollison, 1948), on Rossi-Cholodny slides (Jensen, 1934; Starkey, 1938), on stained soil plates (Warcup, 1951a; Stenton, 1953), in oil-water suspensions of soil (Ledingham and Chinn, 1955), and in soil suspensions (Dobbs and Hinson, 1960). Warcup (1955b, 1957) investigated the unit of origin of colonies developing on dilution plates prepared from wheat-field soil and found that at least 54–82% of the colonies on the plates grew from spores. This means that in this soil there are of the order of $75–150 \times 10^3$ viable spores/g oven dry soil; one sample gave $1{\cdot}5 \times 10^6$ viable spores/g oven dry soil. These figures may be low since it is known that not all spores in soil enter the suspension. A few float on the surface, others are attached to, or embedded in, the heavier soil particles of the residue; further, individual fragments, either in the suspension or in the residue, may contain more than one spore. Spores observed include sporangiospores, ascospores, conidia and chlamydospores. Data suggest that not all soils may contain such high numbers of fungal spores. Dobbs and Hinson (1960) comment that many spores obtained from an A_2 horizon under beech had angular shapes and were difficult to distinguish from mineral particles even after they had been stained. Many spores in wheat-field soil were also angular, the majority of these appearing to be chlamydospores.

Little is known of the longevity of spores in soil; it is probable, however, that there is great variation in survival of different spores, either of the same fungus or of different fungi. Chinn (1953) and Park (1955, 1956a), who added spores to soil, noted that, even with the addition of decomposable organic materials, most of these spores decomposed rapidly, and those which germinated were either destroyed by the activities of soil organisms or produced chlamydospores. Other reports, however, suggest that spores may survive for long periods in soil. Caldwell (1958) found that a few conidia and many chlamydospores of *Trichoderma viride* survived for over 1 year in garden soil, and Chinn and Ledingham (1958) found that conidia of *Helminthosporium sativum* survived 12–22 months in soil in the field.

While asexual spores are considered primarily organs of dispersal, it is likely that in general dispersal in soil is on a limited scale compared with the possibilities available in aerial habitats. Even so this dispersal may be no less

important in the biology of the organisms concerned. Widespread root attack by *Phytophthora* or *Aphanomyces* following mass dispersal of zoospores under wet conditions may indicate dispersal approaching the scale possible in some aerial habitats, but presupposes a number of substrates suitable for colonization being available at the same time. As emphasized by Garrett (1955), however, fungal habitats in soil are often separated in time as well as in space, and under these conditions survival may be more important than dispersal. Perhaps the comments that Fennell (1960) made in discussing methods of preservation of fungal cultures are pertinent here: "it is perhaps noteworthy that certain genera, *Aspergillus*, *Fusarium*, *Mucor*, and *Rhizopus*, have been preserved by all the techniques considered here. Their ability to survive under a variety of conditions is undoubtedly a measure of their inherent resistance and an explanation of their ubiquity." Species of these genera are common in soil.

Dobbs and Hinson (1953) and other workers (Chinn, 1953; Hessayon, 1953; Jackson, 1958) have reported a widespread mycostatic (Dobbs, Hinson and Bywater, 1960) or fungistatic factor that inhibits germination of fungal spores buried in soil, although such spores may germinate when placed in distilled water. It is possible that the reaction of spores to this factor prevents wastage from germination in the absence of organic matter suitable for supporting growth (Waid, 1960).

Sexual spores of fungi may occur in soil; many of these are resting spores and are capable of surviving long periods. Legge (1952) found that oospores of *Phytophthora cactorum* and *P. megasperma* survived in soil for over a year. *Phytophthora fragariae* may survive in soil at least 13 years (Hickman, 1958). The resting spore of *Synchytrium endobioticum* set free in the soil by decay of infected potato tubers may remain viable for as long as 10 years (Muskett, 1960). Comparatively little is known about the presence or survival of ascospores in soil, and even less on basidiospores.

It is evident that many points of interest on the occurrence and the role of fungal spores in soil still await elucidation.

7. Fructifications

Fructifications vary in size and complexity from simple, scarcely differentiated conidiophores to the complex sporophores of the larger Ascomycetes and Basidiomycetes. The factors inducing the formation of these widely different types of reproductive structures are likely to differ greatly and may be very distinct from those favouring maximum production of mycelium (Cochrane, 1960).

While some large complex fructifications are hypogeous (Endogonaceae, Tuberales and many Gasteromycetes), most occur at the soil surface. Fructifications of agarics, and other large fungi are common in woodlands and pastures; much less is known of their occurrence in agricultural soils. In wheat-field soil resupinate Basidiomycetes may fruit beneath clods (Warcup and Talbot, 1962), but this habitat should probably be considered as an extension of the soil surface. Many fungi have fructifications of a size that

could be formed within soil, in the larger cavities, if not within the finer pores. Dobbs and Hinson (1960) consider that many moulds are able to spore in soil cavities of the order of 200 μ in diameter. A point on which we lack adequate information, however, is the relative importance of sporulation within soil compared with that on the soil surface.

While fructifications have been noted in soil pores (Kubiena, 1938; Warcup, 1957), on Rossi-Cholodny slides (Starkey, 1938; Sewell, 1959b), and on roots and other structures in soil (Tribe, 1957a; Waid, 1960), many workers have commented upon the apparent scarcity of fungal fructifications to be found in soil. In general, studies of soil show copious spores and some hyphae but very few fructifications are seen. Yet the high number of spores would indicate that sporulation must be relatively common unless spores have a considerable life in soil. There are probably several diverse reasons for this apparent lack of fructifications: some are ephemeral; others are so like hyphae that unless spores were present the fructifications would remain undetected. While fructifications usually develop after periods of active growth, growth of a fungus may occur in soil without conditions being suitable for sporulation. This is common in fungi with complex sporophores, but may also occur in moulds. Warcup (1957) showed that many fungi, while present throughout the year, have restricted periods of growth and sporulation in soil. Unless soil is examined at the appropriate time, fructifications of these fungi are not observed.

Another reason for this apparent lack of fructifications in soil may be that examination of the soil surface has been neglected. Many fungi are known to fruit at the soil surface, often on residues, but this area, particularly in agricultural soils, has not been thoroughly explored as a site for fungal fructifications. In arable soil the surface is highly complex with both exposed and protected surfaces. In studies of wheat-field soil (Warcup, unpublished data) many fungi have been found fruiting at the soil surface, particularly but not always on the protected under surface of clods. Fructifications obtained include those of species of *Mortierella*, *Mucor*, *Rhizopus*, *Absidia*, *Cephalosporium*, *Fusarium*, *Trichoderma*, *Penicillium*, *Helicomyces*, *Helicosporium*, *Phymatotrichum*, and Basidiomycetes such as *Thanatephorus*, *Sistotrema*, *Oliveonia* and *Sebacina*. Some fructifications are associated with plant residues but others have no obvious connection. The presence of these fructifications at the soil surface raises many interesting questions on the fate of the spores produced, and on possible spore dispersal by wind, rain or members of the soil fauna, but the answers to these questions are still unknown. At least in the case of *Helminthosporium sativum*, spores formed on residues at the soil surface are known to enter the soil and to survive for long periods (Chinn and Ledingham, 1958).

Fructifications also occur in the wheat-field soil itself, being most frequent in the complex of worm tunnels in the upper 1–3 in of soil. After ploughing and seeding, the loose surface of the soil becomes flattened and compacted during heavy rain. Following ploughing, worm action, mainly by *Eisenia rosea* and *Allolobophora caliginosa*, produces a series of horizontal and

vertical tunnels in the upper layer of the soil where the worms feed on plant residues; these tunnels may remain in the soil until it is ploughed again. Fungal sporulation is often noted in these tunnels, sometimes on the remains of soil animals that use them, sometimes on residues, sometimes on mineral particles. It is interesting that sporulation on buried residues is often confined to those portions which abut on tunnels or other large cavities in the soil. Fungi noted sporulating on buried straws include species of *Dinemasporium*, *Chaetomium*, *Myrothecium*, *Periconia*, *Trichoderma*, *Gonytrichum* and *Brachysporiella*.

Kubiena (1938) made some interesting observations on the fungi fruiting in soil pores and found that, in general, there was a reduction in the size of fruiting structures with decrease in pore size. He also noted that the dimensions of fruiting structures in soil were smaller than those recorded for the same fungi grown in culture; this, however, could be an effect of low nutrient level in soil. In wheat-field soil, an interesting case of effect of pore size was seen with *Rhizopus*. Sporangiophores formed in larger cavities were normal and straight whereas those occurring in smaller cavities were coiled and spring-like (Warcup, 1957).

Some fructifications are embedded in organic particles; this may explain their apparent absence when soil is examined microscopically. Many oogonia (Warcup, 1952; Boosalis and Scharen, 1959), sporangia of chytrids (Willoughby, 1961), and vesicles of endophytes (Nicolson, 1961) are embedded in humus particles, either following breakdown of plant tissue or through being surrounded by a considerable accumulation of bacterial cells and small mineral particles (Barton, 1958), so that their presence would not be suspected without staining or allowing germination.

The fate of spores from fructifications formed in the soil is not known, or whether such fruiting constitutes a major source of supply of spores to the "soil spora."

B. Substrates for Growth of Fungi in Soil

Information on occurrence of fungi "in the soil" is very imprecise, and it has been emphasized that fungal occurrence should be considered in relation to specific substrates or microhabitats rather than to the complex, soil itself (Chesters, 1949, 1960; Garrett, 1951; Harley and Waid, 1955). All fungi are dependent upon organic materials for growth, and since much of the organic matter in soil occurs as discrete units, varying from small humus particles to tree roots, data on occurrence and growth on or in such substrates will further our understanding of fungal life in soil. Fungi may also be able to use humified organic matter, but knowledge of their ability to do so *in situ* is lacking.

While the importance of specific substrates for fungal growth has become recognized, there have been major problems concerned with their study, with determining what organisms are present on a substrate, with the difficulty of differentiating between dormant and active portions of a fungus or of different fungi, and with measuring, in some sense, the "activity" of fungi on

natural substrates. Further, there is usually difficulty in interpretation owing to the complexity of natural substrates, particularly in relation to the small size of the microhabitats that may be inhabited by individual fungal colonies (Griffin, 1960; Burges, 1963). As Stanier (1953) has emphasized, a "single cellulose fibre provides a specialized environment with its own characteristic microflora, yet may occupy a volume of not more than a cubic millimetre." While this is true for many moulds, it should be borne in mind that some Basidiomycetes, such as *Marasmius oreades*, may migrate through soil, occupy a considerable volume, and decompose many different substrates; there are probably many gradations between these two extremes.

Since a substrate is changed by supporting the development of micro-organisms, it may be expected in the course of time to provide a succession of microhabitats. Garrett (1955) has stressed that a succession of micro-organisms does not improve but rather depletes the capacity of a habitat to support further plant life, so that the end point of such a succession is not a persisting climax, as with higher plants, but zero. Some microbiologists have questioned the occurrence of an ordered succession of organisms on a microhabitat within soil because it is technically difficult to demonstrate, but there is evidence that succession of fungi may occur on certain roots (Waid, 1960) and on litter. With simpler substrates, Tribe (1960a) has shown an interesting succession of fungi and other organisms on Cellophane, and Griffin (1960) has recorded fungal succession on hair in contact with soil. Their work shows that while a given substrate in soil may have a characteristic fungal flora and succession, these may vary within wide limits. Apart from physical and environmental factors which may influence succession, the species composition of different soils as providers of fungal inoculum is highly important.

Garrett (1955) remarked that a new picture of the distribution of many soil micro-organisms is slowly emerging. "We may imagine the soil as a three-dimensional pattern of substrates, each of which passes in turn through the successive phases of colonization, exploitation and exhaustion. Sites of former substrates, now exhausted, are marked by the resting cells of organisms that had colonized them, at least for as long as the resting cells survive." Although fungi may grow away from a colonized site, Garrett considers that many form resting cells upon the substrate rather than growing out as mycelium through the soil. Certainly it is possible for fresh substrates to come to a resting fungus in soil, for instance, by growth of roots or by movement of members of the soil fauna.

Since substrates for fungal growth consist of living or dead, fresh or partly decomposed plant or animal tissues on or in soil, the number of possible substrates and microhabitats present in different soils, under different vegetations and in different climatic zones is legion. There is much incidental information on fungal occurrence on different substrates and on the ability of fungi to decompose various materials, particularly under laboratory conditions, but as yet only a few substrates in soil have been studied in any detail. Also the value of such studies is very dependent on the methods used and particularly on the amount of microscopic examination carried out during

the investigation. Study of a substrate by dilution techniques alone is likely to record predominantly the dormant organisms from its surface rather than the fungi inhabiting it (Harley and Waid, 1955; Stenton, 1958). This account of substrates in soil is far from exhaustive but outlines some major groups of substrates, also some of the problems associated with their study.

1. Litter

Chesters (1960) has pointed out that while the organic matter of natural soils reaches its maximum value on and in the surface layers, most mycologists interested in soil-inhabiting fungi neglect the surface litter "in favour of that apparently entrancing and most elusive quantity—the soil." He has emphasized both the importance of surface litter in the economy of natural soils and that little is known of the fungal flora of this layer.

Much work has been carried out on forest litter, particularly in relation to the formation of mull and mor, but comparatively few detailed studies have been made of the fungi of various litter horizons or their relation to decomposition *in situ*. Litter layers, from the surface downwards, are often designated as the litter (L), the fermentation (F), and the humification (H) layers (Hesselman, 1926).

Saitô (1956, 1957, 1958, 1960) studied microbiological decomposition, both by direct observation and by plating methods, in the L and F layers of beech litter. The L layer consisted of freshly fallen leaves and brown leaves only slightly subject to microbial attack; surface leaves were liable to dry out rapidly and thoroughly. In the F layer, where the moisture content remained more constant, many leaves turned yellow following attack by Basidiomycete mycelia associated with a vigorous growth of bacteria. Infected leaves first became much thinner without losing their structure, then mouldy from overgrowth of Basidiomycete mycelia; later, growth of other organisms was seen and leaves gradually became transformed into amorphous debris. Not all leaves in the same layer became infected by Basidiomycetes, furthermore decomposition did not always take place uniformly throughout a leaf. Four Basidiomycetes, including two species of *Collybia* and a *Mycena* fruited on the experimental area.

As is usual in such investigations, plating techniques failed to record Basidiomycetes, but showed that a few species of *Penicillium*, *Absidia*, *Mucor ramannianus* and *Trichoderma viride* were widespread throughout the litter horizons. Saitô found that in yellow, Basidiomycete-infected leaves there was a marked disappearance of lignin followed by a rise in the quantity of water-soluble substances. He considered that the filamentous moulds recorded by plating were active on the water-soluble substances initially present in the leaves and on these materials liberated later during lignin decomposition. Basidiomycete mycelia in the decomposing leaves were finally broken down by bacteria.

Leaf litter of conifers, with its strong mor-forming tendencies and with an accumulated bulk of material at different stages of decay, has been investigated on many occasions. The studies on decomposition of litter of *Pinus*

sylvestris in Delamere Forest, Cheshire (Kendrick, 1958, 1959; Parkinson and Kendrick, 1960; Kendrick and Burges, 1962) are noteworthy in illustrating the different pictures of fungal occurrence obtained by different methods of investigation, in showing how important it is that microbiological sampling be undertaken in relation to the characteristic horizons of soil or litter, and in pointing out the importance of knowledge of the time sequence of the events being studied.

Kendrick found that soil plates and soil dilution plates indicated a population of heavily-sporing moniliaceous fungi such as species of *Penicillium* and *Trichoderma viride* in the litter. Using a modification of the Harley and Waid serial washing technique to remove surface propagules from decomposing needles before plating out, he obtained *Trimmatostroma*, *Pullularia*, *Fusicoccum* and *Desmazierella*, besides species of *Penicillium* and *T. viride*. This result contrasts markedly with that obtained from unwashed needles. Kendrick remarked that *Desmazierella acicola*, perhaps the most important internal colonizer of the needles, was not isolated on dilution plates. Direct microscopic observation revealed that even isolation from washed debris failed to give a full picture of the fungal population, since needles of the L layer were often seen to be colonized by the parasitic *Lophodermium pinastri* (pine needle cast) which persisted and fruited in the litter but did not grow in culture. Furthermore two previously undescribed dematiaceous hyphomycetes, *Helicoma monospora* and *Sympodiella acicola*, and Basidiomycete mycelia were seen in the F layer, but were not isolated from washed debris.

Microscopic examination of the H layer also showed large numbers of dematiaceous hyphal fragments, an observation previously made by Romell (1935). These fragments invariably failed to grow by hyphal isolation which suggested that they might have been produced, at least in part, in the overlying layers. It was found that many of the hyphal fragments consisted primarily of empty pieces of conidiophores of *Desmazierella acicola* from the L layer. These had been attacked by oribatid mites and many of the hyphal fragments were originally enclosed in faecal pellets deposited in the H or A_1 horizon. Later these pellets had disintegrated and released the empty conidiophore fragments (Kendrick and Burges 1962).

Summarizing the results obtained by various methods, Kendrick and Burges (1962) suggest the following sequence of decomposition of needles of *Pinus sylvestris* at Delamere. In spring about 40% of living needles on a tree become infected by *Lophodermium pinastri*, a parasitic Ascomycete, which produces no obvious symptoms at this stage. Other fungi present but without visible symptoms include *Coniosporium* sp., *Pullularia pullulans* and *Fusicoccum bacillare*. The latter two appear to attack needles which are senescent and which generally die and become brown while still attached to the tree. At this stage 80% of these needles are infected with *Pullularia* and 90% with *Fusicoccum*.

These brown needles fall to the ground in late August or in early September, and become part of the L layer of litter, in which they spend some 6 months.

During this time the pycnidia of *Fusicoccum bacillare* and the hysterothecia of *Lophodermium pinastri* are produced, providing the inoculum whereby further living needles on the tree become infected. Meanwhile many of the needles on the ground are invaded by *Desmazierella acicola*, a discomycete, which was recorded only in its *Verticicladium* conidial state. Six to eight months after they had fallen, *Verticicladium* was recorded from 70–100% of the needles.

The next stage of the succession, represented spatially by the F_1 layer, takes about 2 years. During this phase colonization proceeds along two distinct lines. *Desmazierella acicola* attacks the interior of needles where it lays down zones of black pigment and each summer produces numerous conidiophores through stomata. Meanwhile the exterior of the needle, now surrounded by a much more humid atmosphere than that found in the surface litter, is colonized by the Hyphomycetes, *Sympodiella acicola* and *Helicoma monospora*. These fungi produce appressed networks of fine dematiaceous hyphae, and later enormous numbers of small darkly pigmented conidiophores are formed. External and internal colonizers combine to bring about the darkening in colour which is such a characteristic feature of the F_1 layer.

After about 2 years in the F_1 layer, the character of the needles changes once more. They become tightly compressed and generally break up into fragments. Those fragments which have been attacked by fungi are gradually penetrated by members of the soil fauna which are the dominant organisms of this phase. Mites, Collembola, enchytraeid worms and others remove the innumerable conidiophores of *Helicoma* and *Sympodiella* while mite instars eat their way through the interior of the needles, eventually bringing about complete comminution. Those fragments which have escaped extensive internal attack by fungi accumulate in the F_2 layer. *Trichoderma viride* and one or more species of *Penicillium* occur on most of these but these fungi are usually present only as a high spore potential.

The needle fragments are slowly attacked by Basidiomycetes and a sterile dematiaceous form over a period of about 7 years. Eventually the meiofauna complete their physical reduction also, and the remains enter the H or humus layer. Here biological activity seems to be at a very low level.

The studies of Witkamp (1960) on decomposition of oak litter emphasize the role of Basidiomycetes in litter breakdown, also the importance of members of the soil fauna in the comminution and decomposition of litter. Witkamp and Van der Drift (1961) have also considered breakdown of oak litter in relation to environmental factors and to time.

2. *Roots*

Roots provide a major habitat or series of habitats for fungi living in soil, but discussion of roots lies outside the scope of this contribution except to note that they provide the main source of plant residues in some soils.

3. *Plant residues*

It is well known that addition of plant residues to soil greatly stimulates the fungal population. There have been, however, few detailed studies of

fungal decomposition of such added material. The ability of root-infecting fungi to survive in or to colonize residues has attracted much attention (Garrett, 1956) but usually without detailed study of other organisms present or of the course of decomposition. Martin, Anderson and Goates (1942) added a considerable amount of plant residues to soil and traced the rise in activity of fungi and the disappearance of constituents of the residues. During the first period Phycomycetes, including *Mucor* and *Rhizopus*, increased in number, and sugar and starch were rapidly decomposed. Then followed a rise of *Pencillium* and *Aspergillus*, coupled with a loss of cellulose and hemicellulose, and lastly a rise of other fungi coupled with decomposition of lignified material. This study, however, was made by the soil dilution plate technique, which would have failed to record any non-sporing mycelia decomposing the added residues.

More recently several workers have studied a later stage of decomposition of residues and have washed debris particles from soil and studied their fungal populations. Most data show that different fungi are recorded from washed than from unwashed particles, there being, in general, a decrease in the frequency of isolation of heavily-sporing forms (Parkinson and Kendrick, 1960). This observation has been considered to support the contention that many of the isolates obtained from washed substrates are from mycelium. It is noticeable, however, that many of the fungi obtained are organisms known to produce resting spores, oospores, sclerotia or chlamydospores, in decomposing tissues; they include *Aphanomyces* and *Rhizoctonia* (Boosalis and Scharen, 1959), *Fusarium*, *Pythium* and *Cylindrocarpon* (Parkinson and Kendrick, 1960; Parkinson and Williams, 1961; Nash *et al.*, 1961). Much more information is necessary before we can be sure that these fungi are active in decomposition of debris particles in soil.

The studies of Tribe (1957a, 1960a, b, c) on the organisms colonizing Cellophane film are of interest, although this is not a natural substrate. Cellophane is a pure regenerated cellulose and its advantages are its simplicity as a substrate and its transparency, which renders it excellent for microscopic examination. Tribe has shown that relatively few fungi in a soil are capable of attacking cellulose film, and that many of these are species which are not recorded in dilution plate studies. The fungi attacking Cellophane differed markedly from soil to soil. In England some alkaline soils gave *Botryotrichum piluliferum* (*Chaetomium piluliferum*; Daniels, 1961), *Chaetomium*, *Humicola grisea*, chytrids and occasionally *Stachybotrys*. *Stysanus* was common in a chalk soil and *Oidiodendron* in an acid soil. A brown forest soil in Canada yielded *Rhizoctonia*, *Humicola*, *Botryotrichum* and chytrids. Some fungi grew on the surface of the film; others including species of *Humicola* and *Botryotrichum*, were notable for their "rooting branches" in the thickness of the Cellophane.

4. Seeds

While much is known of the fungi which are seed-borne, comparatively little attention has been paid to germinating seed as a substrate for the growth

of fungi in soil. Seed may liberate sugars, amino acids and other substances during germination; further, all reserve foods may not be utilized by the developing germling. Warcup (1957) found that *Rhizopus* often grew on germinating wheat seed after emergence of the shoot above ground. Although *Alternaria*, *Stemphylium* and *Cladosporium* were common on wheat seed they were not observed to grow extensively on seed planted in the field. *Fusarium*, in part seed-borne, *Penicillium*, *Rhizopus* and occasionally *Thraustotheca clavata* often grew extensively and the dark synnemata of *Podosporiella verticillata* which may be seed-borne (Wallace, 1959) were also occasionally found (Warcup, unpublished data). The latter fungus was more frequent on seed of weed grasses than on wheat. Detached pieces of synnemata are also not uncommon among the heavier soil particles concentrated for hyphal isolation.

5. Animal substrates

Apart from the group of predacious fungi which attack nematodes, protozoa and amoebae, very little attention has been paid to animal tissues or residues as substrates for fungi in soil. This is perhaps surprising, particularly with groups such as mites and collembola, which may occur in soil in high number; similarly cast insect exuviae often occur in sufficient number to provide adequate material for study of organisms able to use this source of chitin in soil. There have been incidental observations, particularly of species of *Aspergillus* and *Penicillium* fruiting on dead soil animals (Warcup, 1957; Sewell, 1959b), but as far as I am aware, no systematic study of the occurrence and growth of fungi on members of the soil fauna, apart from nematodes, has been made. Insect parasites, such as *Entomophthora* (Miller *et al.*, 1957; Griffin, 1960) and *Beauveria bassiana*, have been isolated from soil itself, and the latter was of frequent occurrence in certain profiles (Sewell, 1959a; Brown, 1958b). Little is known, however, of the biology of these fungi in soil; similarly little is known of the life of the species of *Cordyceps* that infect soil-inhabiting insects.

The predacious fungi form a well-marked ecological group, united by their habit of capturing and consuming minute animals. Taxonomically they fall into two groups: the Zoopagales, belonging to the Zygomycetes; and the predacious Hyphomycetes, a diverse group in the Moniliales. Two general forms of predacious activity may also be recognized in members of both taxonomic groups. The endozoic forms such as *Endocochlus* and *Harposporium* pass the whole of their vegetative state within the bodies of their hosts, which they usually attack by means of sticky spores. The second group consists of active predators such as *Stylopage* and *Arthrobotrys*, which capture living animals on their mycelia. The best-known predacious fungi are the nematode-trapping Hyphomycetes with their remarkable array of specialized trapping devices, sticky knobs, nets, non-constricting and constricting rings. Knowledge of these fungi has been particularly due to the studies of Drechsler, who isolated many predacious fungi from leaf mould. They are also widely distributed in soil, although there is little direct evidence of their

activity in this habitat since most observations on predacious fungi have been made from agar cultures. Capstick, Twinn and Waid (1957) showed, however, that a small proportion of free-living nematodes isolated directly from forest litter were infected with various predacious fungi thus confirming that these organisms are active in a natural habitat. Cooke (1962), using a direct method of observation, has studied the behaviour of nematode-trapping fungi during decomposition of organic matter in soil in the laboratory.

Fungi also attack nematode eggs. Van de Laan (1956) investigated the fungi which attack eggs and kill the larvae within the cysts of *Heterodera rostochiensis*. Certain fungi, including *Phialophora heteroderae*, *Monotospora daleae*, *Phoma tuberosa* and *Colletotrichum atramentarium* were found repeatedly in or on cysts. The latter two fungi also occur on potato. Cysts from Jersey in the Channel Islands contained *Penicillium vermiculatum* and *Pseudoeurotium ovalis*. Ellis and Hesseltine (1962) have recorded that *Rhopalomyces* is parasitic on nemotode eggs. *Fusarium* and *Cephalosporium* may be parasitic (Lýsek, 1963) on eggs of parasitic roundworms (*Ascaris* sp.).

Griffin (1960) studied the colonization of hair by fungi in soil in the laboratory. Although hair had previously been widely used as a bait for keratinolytic fungi, no previous study of its general colonization had been made. Besides keratin, hair contains traces of many other substances (Bolliger and Gross, 1952). Griffin found that many fungi sporulated on the surface of hair so that direct observations could be used as a check on isolation data. Initial colonizers were often Fusaria, certain Penicillia, and various members of the Mucorales; these were overlapped or followed by a second group including *Chaetomium cochlioides*, *Gliocladium roseum*, *Humicola* species and certain other Penicillia; the final group comprised keratinolytic fungi such as *Keratinomyces ajelloi* and *Microsporum gypseum*. Griffin remarked that while the broad outlines of the general succession were clear, the fate of individual colonies as they were succeeded was uncertain. Many sporulated profusely as they passed their peak of activity and then survived as dormant spores, the hyphae remaining at least initially as empty tubes. In others, however, much of the protoplasm of the colony was probably destroyed by direct parasitism of a succeeding fungus.

Griffin also commented that the existence of keratinolytic fungi in the great variety of soils which have now been examined by various workers raises a problem of great interest, for it seems scarcely possible that the accession of keratin in normal soils should be sufficient to support an almost ubiquitous keratinolytic flora, even allowing for very prolonged periods of dormancy. Pugh and Mathison (1962) found keratinophilic fungi to be common in some salt marsh and sand dune soils, particularly from sites where there was a natural deposition of keratinous substrates such as rabbit fur and bird feathers. Mathison also suggested that keratinophilic fungi compete successfully with other fungi on a wide range of protein substrates, particularly fibrous proteins which are normally fairly resistant to digestion by proteolytic enzymes. Watling (1963) has found keratinophilic fungi to be present in dung of birds of prey.

6. Fungal structures

Fungal structures themselves may provide substrates for other fungi. A number of soil fungi appear capable of parasitizing other fungi. Fructifications of agarics are frequently attacked by fungi including Mucorales, Fungi Imperfecti and other agarics. Below ground, *Scleroderma* may be infected with *Boletus parasiticus*; the ascomycete *Elaphomyces* may be parasitized by *Cordyceps*. Hawker (1955b) recorded how *Sepedonium chrysospermum* occurred on fruit bodies of *Melanogaster* and *Rhizopogon* and extended and sporulated profusely in the soil some centimetres from the host. *Syncephalis* may parasitize *Mortierella* particularly where the host is growing abundantly (Warcup, 1952).

The behaviour of *Trichoderma viride* as a parasite of other soil fungi has attracted much attention (Rishbeth, 1950; Aytoun, 1953; Boosalis, 1956; Campbell, 1956) since its original discovery by Weindling (1932). A *Papulospora* (Warren, 1948) and *Penicillium vermiculatum* (Boosalis, 1956,) have been recorded as parasites of *Rhizoctonia solani*; *R. solani*, itself, has been recorded as parasitic on several Phycomycetes (Butler, 1957). Campbell (1947) isolated *Coniothyrium minitans* from sclerotia of *Sclerotinia trifoliorum* and showed that it was capable of killing sclerotia. Tribe (1957b) showed that *C. minitans* was of frequent occurrence in East Anglia and that spores of the parasite may persist ungerminated for at least a year in disintegrated sclerotia in soil; so far the fungus has only been found in connection with sclerotia.

Fungi may also take part in decomposition of dead fungal structures, although little information is available. Since some fungi possess chitinases it is probable that they may decompose chitin-containing hyphae. Although the occurrence in soil of organisms which decompose chitin has been studied (Veldkamp, 1955; Witkamp, 1960; Y. Lingappa and Lockwood, 1961), little is known of the ability of these organisms to utilize either fungal or insect chitin in soil.

IV. FACTORS AFFECTING GROWTH OF FUNGI IN SOIL

Chesters (1949) commented that, while much information has been collected on fungi in soil, so far only a very indistinct picture of the fungus at work has been obtained. Much of the difficulty in such study centres around the question of having adequate methods for distinguishing between active and resting structures of fungi in soil. In several investigations such difficulties have been overcome, at least in part, by combinations of microscopic examination and isolation procedures. While many points still await elucidation, it is perhaps now possible to sketch some outlines of fungal life in soil.

A. Fungal Propagules in Soil

Much work has indicated that soil contains a large number of inactive units of fungi; these include "resting hyphae," chlamydospores, sclerotia,

sporangiospores and conidia, oospores, ascospores and probably, though there is little information on this point, basidiospores. The number of resting units varies markedly with the soil, with its past history and with the kinds of organisms present. In Urrbrae loam, some Basidiomycetes such as *Waitea circinata* are present as 1–10 sclerotia per 500 g of soil, others such as *Omphalina* may reach concentrations as high as 20–25 sclerotia per g in some areas; at the other end of the scale certain Penicillia are present relatively uniformly in the surface layers as $1–4 \times 10^4$ spores per g of oven-dry soil.

It is now considered that, in general, the "soil spora" (the population of chlamydospores, sporangiospores, ascospores and conidia) provides the bulk of the propagules in soil suspensions and hence on soil dilution plates, that they are common on the surface of roots and other particles in soil and represent the majority of the units obtained in rhizosphere studies (Harley and Waid, 1955). While we know of the existence of the soil spora, many facts concerning the formation, occurrence and longevity of its members are not yet known.

1. Mycostasis

Since the sporangiospores and conidia of many fungi occurring in soil are able to germinate in distilled water, the presence of large numbers of ungerminated spores in soil may appear anomalous. Dobbs and Hinson (1953), however, reported widespread inhibition of fungal spores in the organic layers of most soils. Failure of spores to germinate when in contact with soil has been observed in many soils in different parts of the world (Chinn, 1953; Hessayon, 1953; Park, 1955; Chinn and Ledingham, 1957; Jackson, 1957, 1958a, b; Dobbs *et al.*, 1960).

Dobbs and Hinson used cellulose film to demonstrate inhibition of spore germination in soil, the most sensitive test being the "closed" film test where spores of the fungus are placed inside folded film pressed between two lumps of soil (Dobbs *et al.*, 1960). Free margins of the fold act as controls. Other tests for inhibition are based on the use of agar; Jeffreys and Hemming (1953) placed agar slabs directly on the soil profile; Chinn (1953) coated a slide with spores in very weak agar; Molin (1957) and Jackson (1958a) have used agar disks. More recently, Lingappa and Lockwood (1963) have placed spores directly on a compacted soil surface. After time for germination spores are recovered in a collodion film.

Dobbs and Hinson (1953) found that germination of *Penicillium frequentans* spores in contact with fresh moist surface soils was totally inhibited. Inhibition decreased with depth and appeared to coexist broadly with the region of biological activity in soil. No fungus whose spores germinate readily in distilled water was found to be unaffected. Inhibition is removed by severe heating or by desiccation, and is masked by adding glucose above a threshold quantity which varies widely from soil to soil, also by any other treatment liable to release nutrients by killing soil organisms, by organic solvents (ether, acetone) and by mixing soil with activated charcoal. Inhibition usually returns after the glucose or other nutrient has been removed from the soil.

Neither the nature of the inhibitor nor whether it is a complex rather than a single substance is known. Dobbs *et al.* (1960) consider that it might be an anti-metabolite. Brian (1960) thought that there were still too many uncertainties concerning mycostasis to know whether it is a positive effect. Park (1960, 1961b) has compared mycostasis with the staling of fungal cultures on media. Lingappa and Lockwood (1961) consider that the indirect techniques used to demonstrate fungistasis do not reveal the presence of fungistatic substances in soil, but provide a substrate for growth of micro-organisms which produce antibiotic substances thus making the assay medium fungistatic. Since fungal spores do not germinate in soil it is considered that nutrients diffusing from spores stimulate a microflora around them with resultant inhibition of their germination. Dobbs and Carter (1963) consider that there is so far no evidence that soil mycostasis is occasioned, to any large degree, by the proliferation of inhibitory organisms on the actual spore surface, but there is some evidence that fresh spores may stimulate the growth of such organisms in their vicinity, with the production of diffusible inhibitors in the soil solution. Griffin (1962) and Lockwood and Lingappa (1963) found that both antagonistic and non-antagonistic micro-organisms induced an observable fungistatic effect in non-amended autoclaved soil. They consider that the fungistatic effect of natural soil may result in part from the general saprophytic activities of the soil microflora.

Some soils possess inhibiting factors whose properties differ from mycostasis (Dobbs *et al.*, 1960). Jeffreys and Hemming (1953) detected traces of antibiotic activity in soil, their observations suggesting discontinuous "islands" of inhibition rather than a widespread "sea" of inhibition. Lingappa and Lockwood (1962) found that certain lignin-like substances isolated from soil were fungistatic.

Apart from observations on hyphal growth of some Basidiomycetes (Molin, 1957; Dobbs *et al.*, 1960), there is little information on the effect of mycostasis on other fungal structures in soil. Certainly many sclerotia remain ungerminated in soil but whether they are affected by mycostasis is not known. Coley-Smith (1960) showed that sclerotia from 6-week-old cultures of *Sclerotium cepivorum* would germinate only if the rind had been artificially damaged by abrasion. When unabraded sclerotia were buried in the field for a month or longer, however, some became capable of germination. Germination of sclerotia which produce fructifications may be highly seasonal, as in *Sclerotinia camelliae*, but not in others such as *Sclerotinia sclerotiorum* and *Sclerotinia fuckeliana*.

2. Spore dormancy

The significance of dormancy in relation to fungi occurring in soil is not known. Many Phycomycetes form thick-walled resting spores that do not germinate until after a period of maturation; at least part of this apparent resting period represents time required for cytological processes. With some resting sporangia, the thickness of the wall is one of the decisive factors in germination; resting sporangia grown under conditions inducing a thin wall

germinate without a rest period (Cochrane, 1958). Ascospores of many Ascomycetes, particularly but not exclusively coprophilous species, exhibit a pronounced dormancy which is often broken by brief exposure to heat; Warcup and Baker (1963) found that certain soils contain large numbers of ascospores which do not germinate on agar unless the soil has been heat-treated. In culture oospores of *Phytophthora cactorum* exhibit dormancy (Blackwell, 1943), but Legge (1952) showed that its oospores buried in soil may germinate after a week or may survive ungerminated for at least a year. In decomposing roots containing copious oospores of *Pythium* I have noted that the fungus was isolated only from chlamydospores present in the material. Whether these oospores were dormant was not tested.

B. Spore Germination

While some sclerotia are nutritionally independent, there is increasing evidence that spores and resting structures of fungi in soil germinate only when stimulated by an external source of nutrients. In some cases this occurs in response to a specific substance, in other cases the response is more general. Known sources of nutrients are seeds, roots and decomposing plant and animal residues. Germination also depends on suitable pH, carbon dioxide concentration, temperature, oxygen and water supply and absence of inhibitors (Cochrane, 1958).

Exudates from plant roots have been shown to stimulate germination of fungal propagules in soil; much of the work has been done with root parasites, but stimulation of saprophytic fungi by roots has also been demonstrated. Germination of spores of *Plasmodiophora brassicae* is stimulated by host and non-host roots (Macfarlane, 1952); *Spongospora subterranea* is stimulated by roots of the host family Solanaceae (White, 1954); banana roots stimulate germination of spores of *Fusarium exysporum* f. *cubense* in soil extracts (Stover, 1958); oospore germination of *Aphanomyces euteiches* is stimulated by roots of peas, soyabean and sweet corn, but significantly greater numbers germinate near peas than near the non-host plants (Scharen, 1960; Cunningham and Hagedorn, 1962); oospore germination of *Pythium mamillatum* is stimulated by exudates from turnip seedlings (Barton, 1957); chlamydospores of *Fusarium solani* f. *phaseoli* germinate most consistently close to germinating bean seed and root tips (Schroth and Snyder, 1961) but germination also occurs near non-susceptible plants (Schroth and Hendrix, 1962). Coley-Smith (1960) found that in the presence of host roots the sclerotia of *Sclerotium cepivorum* (white rot of onion) were strongly stimulated to germinate, there being little or no germination in the presence of non-hosts or in soil alone. Germination was greatest in the root tip region and was independent of contact between roots and sclerotia, occurring over a distance up to 1 cm from the roots. Tichelaar (1961) recorded that root exudates of *Gladiolus* also stimulate germination of sclerotia of *S. cepivorum*. Jackson (1957, 1960) found that conidia of *Gliocladium roseum*, *Fusarium* sp. and *Paecilomyces marquandii* on glass slides buried beneath peas germinated in the immediate vicinity (1–2 mm) of the young roots but not elsewhere, except that some

Fusarium conidia distant from the roots germinated to form chlamydospores. Germ tubes of *G. roseum* and *Fusarium* showed strong tropic growth towards the roots.

There are several records of fungal germination being stimulated by addition of residues to soil. Mitchell, Hooton and Clark (1941) found that organic matter stimulated germination of sclerotia of *Phymatotrichum omnivorum* in the absence of living host roots. Chinn, Ledingham, Sallans and Simmonds (1953) found that the addition of soyabean meal to soil stimulated germination of conidia of *Helminthosporium sativum* present in soil. Further studies showed that wheat-germ bran, oil meals, molasses or green plant tissue were very effective, but that refined flour and commercial sugar were only slightly so, and that dead wheat roots and straw gave no spore germination (Chinn and Ledingham, 1957). Toussoun and Patrick (1962) found that chlamydospores of *Fusarium solani* f. *phaseoli* germinated around decomposing residues of barley, broccoli and beans, and when water extracts of these residues were added to soil.

Factors affecting the germination of spores of the larger Ascomycetes and Basidiomycetes are unknown. Spores of some of these fungi have proved difficult to germinate in culture, but some germinate more readily in the presence of tissues from sporophores. Germination of spores of *Agaricus campestris* has been found to be stimulated by an olefin, 2–3 dimethyl-1-pentane (McTeague, Hutchinson and Reed, 1959).

C. Hyphal Growth

Following germination, fungal growth is dependent upon a suitable substrate or a continuing supply of nutrients, otherwise lysis of germ tubes takes place or the fungus forms a resting structure such as a chlamydospore, and ceases further growth. Continuing growth also depends upon a suitable physical environment.

In general, fungal growth is affected by both high and low temperatures. Various reports (Lebeau and Cormack, 1956; Lebeau and Logsdon, 1958) that fungi can cause damage to snow-covered plants are evidence that certain species grow at low temperatures. The distribution of *Phymatotrichum omnivorum*, however, is limited by low winter soil temperature in conjunction with soil type (Peltier, 1937; Ezekiel, 1945). Waid (1960) reported that mycelial activity on buried nylon gauze was less in winter, when temperature was low, than in summer. Similarly, *Trichoderma viride* is not active in soil at low temperatures (Rishbeth, 1950; Griffiths and Siddiqi, 1961). At high temperatures, so long as water is not limiting, some fungal growth may occur, since many soils contain thermotolerant fungi such as certain species of *Chaetomium* and *Aspergillus fumigatus*.

When the water content of a soil either falls below the wilting point or becomes sufficiently great to impede soil aeration there is great reduction in the active growth of fungal hyphae. Warcup (1957) found negligible activity in an Australian wheat-field soil during the summer dry period when soil

moisture fell markedly below wilting point (30–50% r.h.). While many fungi have been found in waterlogged or periodically inundated sites (Boswell and Sheldon, 1951; Stenton, 1953; Pugh, 1960), there is little information on mycelial activity in such soils.

In his admirable review on soil moisture and the ecology of soil fungi, Griffin (1963) considers that it is probable that nearly all fungi are able to exert the necessary force to absorb water and to grow unimpeded by reduced hydraulic conductivity of the soil throughout the suction range pF 0–4·2 and even in drier soils. In soils drier than permanent wilting point, however, ability will be likely to differ from one species to another, probably leading to ecological diversification. Griffin considers that except below permanent wilting point it seems unlikely that the actual volume of water in soil, taken as a factor in itself, has any effect on fungal ecology. While factors such as moisture content, soil texture and structure have a profound influence on fungal activity, it seems that aeration is, in fact, the effective agent.

Fungi are commonly considered to be strictly aerobic and this opinion is basically correct; however, many are able to grow at low oxygen tensions. This ability to grow at reduced oxygen tension is probably important in growth in a subterranean environment. However, although the concentration of oxygen in the soil atmosphere is normally about 20%, great reductions can occur in waterlogged or compacted soils and in water films adjacent to respiring tissues, owing to the extremely low solubility and rate of diffusion of oxygen in water. Greenwood (1961) has shown that in a clay loam, no oxygen at all was present at the centre of water-saturated crumbs of more than 3 mm radius, even when the intercrumb space was air-filled. Griffin (1963) remarks that the presence of such crumbs must be fairly common even in well-drained soils and these anaerobic pockets must act as fungal inhibitors as well as providing niches for anaerobic bacteria. Thus oxygen deficiency probably causes a restriction in fungal activities not only in water-saturated soils, where Scott and Evans (1955) have shown that oxygen rapidly disappears, but also in apparently well-aerated soils where individual crumbs are water-saturated. In the former case fungal activity is probably inhibited, whereas in the latter case hyphae will be able to ramify in the air-filled pores but their depth of penetration into the anaerobic centres of crumbs will depend, amongst other factors, on the rate of transfer of oxygen along the length of the hypha.

That carbon dioxide concentration can effect fungal activity is suggested by several workers. Burges and Fenton (1953) studied the effect of carbon dioxide and oxygen on the growth in culture of several fungi isolated from various horizons of a soil. Their results show that fungi abundant near the surface were intolerant of carbon dioxide whereas those abundant in subsurface horizons could tolerate concentrations as high as 10%. Durbin (1959) studying clones of *Rhizoctonia solani*, found that those clones isolated from an aerial environment were less tolerant of carbon dioxide than isolates from subterranean habitats while clones from the soil surface were intermediate in tolerance. Papavizas and Davey (1961b) found that accumulation of carbon

dioxide in the immediate neighbourhood of buried substrates reduced or prevented growth of *Rhizoctonia* into them. Garrett (1937) had earlier suggested that absorption of carbon dioxide by alkaline soils was the factor which increased the growth of *Ophiobolus graminis* in them. All who have investigated the direct effect of carbon dioxide on fungal growth have shown that, although there is considerable variation from species to species, in general the concentration needed to produce appreciable reduction in linear growth rate is of the order of 10–20%. There are indications, however, that change in linear growth rate may be an insensitive measure of the effect of carbon dioxide; for instance, Burges and Fenton (1953) showed that growth as measured by increase in mycelial weight may be markedly reduced at much lower concentrations of the gas. While the volume of carbon dioxide reported from bulk samples of soil atmospheres is usually between 0·2 and 2%, higher values, 10% or more, have been recorded in soil at depth, or after rain or waterlogging (Russell, 1961).

Soil pH affects the availability of plant nutrients but whether this is important for fungi in soil is not known. Some fungi can tolerate a wide range of soil pH, others are restricted to either acid or alkaline environments (Warcup, 1951a; Brown, 1958b). Cowley and Wittingham (1961) have suggested that tannins may be one of the factors influencing distribution of certain species of fungi in soil. They noted that tannin exerted a pronounced inhibiting effect on microfungi from prairie soils, whereas it had no adverse effects on a majority of the species either prominent in forest soils or common to both.

D. Fungal Growth Patterns in Soil

The question of hyphal growth in soil is a complex problem on which our information is very fragmentary. For instance, it is apparent that there is great diversity in growth patterns of fungi in soil; in particular the life of mycelia of many Ascomycetes and Basidiomycetes that form complex fructifications seems to be very different from that of the sporing moulds. Growth rates of the former are often slower and hyphae appear to function for a much longer period; but even between members of each group there is considerable variation. Warcup (1957, 1959) found that in soil some moulds such as *Rhizopus*, *Mortierella* and *Penicillium* have a short life span (3–4 days) from spore germination to formation of spores, whereas some Basidiomycetes may be present in soil as mycelium for several years without forming fructifications. While these Basidiomycete mycelia are perennial, there is little information on the longevity of individual hyphae. It is of interest that Schütte (1956) found that hyphae of moulds such as *Aspergillus* and *Penicillium* were unable to translocate whereas hyphae of the Phycomycetes and Basidiomycetes tested were able to do so.

1. Moulds

Direct examination indicates that different fungi exploit soil and soil habitats in different ways. For instance, Phycomycetes such as *Pythium* and

Mortierella appear to have short-lived mycelium in soil. One can often see phycomycetous hyphae with densely cytoplasmic tips, but a short distance back the cytoplasm becomes vacuolate, and still further back the hyphae appear empty. On the other hand phycomycetous endophytes possess hyphae that ramify from humus particle to humus particle and appear relatively long-lived and resistant to decomposition. With other Phycomycetes such as *Phytophthora* there is doubt whether free growth in soil occurs (Hickman, 1958). Oospores occur in soil, often being embedded in plant residues; germination takes place by the formation of germ tubes which quickly give rise to sporangia (Legge, 1952); the zoospores are attracted to plant roots (Zentmyer, 1961).

Some parasitic Fusaria have also been shown to have limited free growth in soil. The studies of Snyder and co-workers on *Fusarium solani* f. *phaseoli* are of interest; there have been few comparably detailed studies on the biology of saprophytic fungi in soil. *F. solani* f. *phaseoli* is a cortical root- and stem-rotting fungus pathogenic to bean, which it normally attacks by multiple infections of roots and hypocotyl (Toussoun, Nash and Snyder, 1960). Direct examination showed that the fungus exists in soil as thick-walled chlamydospores many of which are embedded in plant debris or particles of organic matter. When macroconidia are added to unsterilized field soil they form chlamydospores either directly or at the end of a short germ tube. Chlamydospores are also formed abundantly at the surface or in the outer cortex of lesions when infected plants die (Nash *et al.*, 1961). Macroconidia are formed at the soil surface in the presence of light (Christou and Snyder, 1962). Although chlamydospores will germinate in water, they were rarely observed to germinate in unsterilized soil, even when the soil was saturated with water (Toussoun and Snyder, 1961). They germinated, however, when in close proximity to germinating bean seed and root tips. Mature roots had little effect on chlamydospore germination and growth when tested in soil. Exudation of amino acids and sugars was most abundant from germinating bean seeds and root tips, and only traces of exudate were detected from mature roots unless they were dried or injured. Solutions of aspartic acid, asparagine, glucose and sucrose stimulated germination and growth of chlamydospores when tested *in vitro*. All these materials were identified as constituents of bean exudate (Schroth and Snyder, 1961). Chlamydospores also germinate near seed of several non-hosts (Schroth and Hendrix, 1962) and around decomposing residues of barley and broccoli, and when water extracts of these residues are added to soil (Toussoun and Patrick, 1962). Data suggest that growth of *F. solani* f. *phaseoli* in soil is limited to the rhizosphere of bean plants, various non-hosts and other temporary supplies of nutrients.

While saprophytic species of *Fusarium* may possibly grow more extensively in soil, it is probable that many features in the growth cycle of *F. solani* f. *phaseoli* apply also to these species. It is known that most *Fusarium* units in soil are chlamydospores (Warcup, 1955b; Nash *et al.*, 1961); *Fusarium* is common in the root zone of various plants (Simmonds and Ledingham, 1937; Waid, 1957, 1960; Parkinson and Chesters, 1958; Peterson, 1958);

macroconidia are often formed on plant residues at the soil surface; conidia or hyphae of many Fusaria added to soil form chlamydospores (Jackson, 1960; Newcombe, 1960); *Fusarium* is often isolated abundantly from debris particles in soil (Parkinson and Kendrick, 1960; Parkinson and Williams, 1961).

It is possible that *Cylindrocarpon radicicola*, which is common on root surfaces (Peterson, 1958; Parkinson and Clarke, 1961; Papavizas and Davey, 1961c; Kubíková, 1963), forms chlamydospores, and is abundant in organic fragments (Parkinson and Williams, 1961), may have a similar life cycle in soil to *Fusarium*. *C. radicicola* is also known, however, to grow on the surface of nematode eggs (van der Laan, 1956).

There is evidence that many other fungi, including species of *Penicillium* and *Aspergillus*, do not normally grow extensively through soil. Sewell (1959b), investigating fungi in a *Calluna*-heath soil, observed Penicillia on few soil slides but in each case these fungi were confined to specific substrates, frequently animal remains, from which there was negligible mycelial spread but considerable sporulation. Discussing growth patterns of fungi in soil, Burges (1960) outlined the *Penicillium* pattern as follows: "A small piece of substrate is densely colonized by the fungus. Spore production occurs heavily over the surface of the substrate, and there is no extension of the mycelium into the surrounding soil." Sewell's observations substantiate this, but other data suggest that not all Penicillia may have the same growth pattern. For instance, *P. vermiculatum* is recorded as a parasite on fungal hyphae in soil (Boosalis, 1956). In Urrbrae loam some Penicillia grow and sporulate on dead soil animals, much as outlined by Sewell; others have been noted on insect exuviae where growth and reproduction are very sparse; others have been noted on the surface of living roots, where once again growth was very sparse and where conidial heads were depauperate compared with those produced by the fungi on isolation in culture; on the other hand, *Penicillium urticae* occasionally flourishes on pieces of root and other substrates in soil and may grow out and sporulate abundantly a cm or so from the substrate. Burges (1963) quotes *Desmazierella* sp. as an example of a fungus occurring in a very restricted habitat, the inside of a pine needle.

With many fungi found in soil, such as coprophilous species, entomogenous fungi, and human and animal pathogens, there is little information available concerning possible growth in soil. Macroconidia of several dermatophytes have been isolated from soil and since these spores are not produced in infected hosts, their presence in soil suggests that they were formed there by the fungus (McDonough *et al.*, 1961). Likewise there is little information on yeasts in soil (Miller and Webb, 1954).

While they are laboratory, not field studies, the observations of Tribe (1957a, 1960a, b, c, 1961) on the decomposition of Cellophane film in soil are of considerable interest. Tribe found that the primary colonizers of buried film were fungi. Chytrids were frequent early colonists but filamentous fungi appeared at the same time. After a short period (2–6 weeks) bacteria developed profusely around the mycelium and over the Cellophane; bacteria were not usually prominent before fungal growth occurred. The bacteria invariably

supported a population of nematodes and sometimes patches of amoebae. Nematodes were often parasitized by predaceous fungi which appeared to be the only fungi capable of developing over the bacterial debris. If no larger members of the soil fauna appeared, colonized film persisted in this condition for several months. Frequently, however, the microbial tissue and cellulose were consumed by soil animals. In acid sand and litter, mites were the predominant organisms; in neutral to alkaline soils Collembola and enchytraeid worms were found. Enchytraeid worms were often seen in the soil from the time of burial of the cellulose film, but did not attack it until it was partly replaced by microbial tissue. Mites and Collembola produced well-defined faecal pellets which contained microbial cells; enchytraeid worms mixed the residues with a large proportion of soil and their excreta were difficult to recognize. Decomposition of mite faeces appeared to be very slow. Went (1959) also found that after Cellophane had been attacked by fungi, mites and springtails would start to eat it.

Different pieces of film varied greatly in the time taken to decompose, even in one soil, and the rate of decomposition was greatly influenced by the organisms present. In the absence of fungi capable of attacking the Cellophane, bacterial growth was slight. Keynan, Henis and Keller (1961) have found that cellulose-decomposing bacteria such as *Cellvibrio* are unable to utilize Cellophane film. Because of the restricted development of their rhizoids chytrids were not of great importance in decomposition unless present in large number. Rooting fungi were more active, but a *Rhizoctonia* was able to reduce film to a mushy condition in 2–3 weeks. Further decomposition was dependent on soil animals consuming the film, the combination that decomposed the cellulose most thoroughly being *Rhizoctonia* and enchytraeid worms. In some soils certain pieces of Cellophane remained untouched for weeks, by which time others were virtually destroyed by *Rhizoctonia* or rooting fungi. Presumably none of these occurred near the untouched pieces and other fungi in contact with them were unable to use this substrate.

Tribe (1961) studied both colonization of cellulose film and its influence on the mineral nitrogen status of the soil. He found that under the conditions of incubation provided, mineral nitrogen in the soil was immobilized within 2 weeks of burial of the film but after 16 weeks nitrate began to be released. There was a general succession of fungi, bacteria, and protozoa, especially thecate amoebae, on the cellulose. He suggested that mineral nitrogen was taken up by soil organisms until a mature population had developed approximately 16 weeks after burial of the film. Thereafter nitrogen was slowly released from the population, which was declining as the cellulose substrate became progressively exhausted. He considered that nitrogenous excretion by the protozoa and autolysis of microbial cells were the biological mechanisms of nitrogen release.

2. *Basidiomycetes and large Ascomycetes*

As with moulds and other fungi with a relatively short mycelial life in soil, Basidiomycetes and Ascomycetes with longer-lived mycelia have many

different growth patterns in soil. These groups, however, have been less extensively studied. Burges (1960) gave the Basidiomycete pattern as: "The fungus colonizes the substrate with a long-lived mycelium and then migrates to other substrates or to a position where it will produce fruit-bodies by means of rhizomorphs or well-developed mycelial strands." The size that such a rhizomorph system may attain is indicated by Grainger (1962), who found by excavation that the length of rhizomorphs from a fructification of *Phallus impudicus* was 55·5 ft. This is probably a general picture of many Basidiomycetes, but other growth cycles are known. A species of *Omphalina* in Urrbrae loam oversummers as sclerotia which are produced abundantly in the late spring (Warcup and Talbot, 1962). In the autumn when the soil again becomes moist the fungus returns to the mycelial condition by germination of some of the sclerotia; only once in the last 7 years have fructifications of this fungus been seen. Several other Basidiomycetes in this soil form sclerotia and may be present in the resting condition for much of the year; their growth cycle has marked resemblances to that of some moulds.

Basidiomycetes that occur in forest litter and soil are considered, in general, to belong to three groups: these are the ectotrophic mycorrhizal fungi which as a group seem incapable of lignin decomposition and only a few are able to break down cellulose; those which produce white rots in wood, causing a break down of cellulose and lignin; and those which produce brown rots in wood, attacking cellulose and hardly affecting lignin (Harley, 1959). It will be noted that these are physiological groups; there is little available information on the ecology of these organisms in soil. Many Basidiomycetes, and particularly mycorrhizal species, are slow-growing and sensitive to competition on agar media, making them difficult to isolate from soil, root tissues or other habitats. Further, it is difficult to identify unknown cultures which may be obtained since data on how to obtain fructifications of Basidiomycetes are scanty.

Melin (1925) showed that several mycorrhizal fungi made their greatest growth in culture upon glucose; more complex carbohydrates were not useful as carbon sources. These results have been generally confirmed (How, 1940; Mikola, 1948), although Norkrans (1949) showed a wider range of utilization of carbohydrates by mycorrhizal species of *Tricholoma*, *T. fumosum* being able to use cellulose to a significant extent. Most mycorrhizal fungi are also characterized by being dependent in culture on the presence of vitamins or growth-factors. Unidentified stimulating substances have also been found naturally: in secretions of germinating pine seeds (Melin, 1925); newly-fallen litter (Melin, 1946); and in root secretions (Melin, 1954; Melin and Das, 1954). The question whether most mycorrhizal fungi are virtually confined to the root zone of host plants or whether they may make appreciable free growth in soil has not been resolved.

Fairy rings are common in woodlands, grasslands and lawns and are the result of activity of soil-inhabiting fungi, mainly Basidiomycetes. Shantz and Piemeisel (1917) classified fairy ring fungi into three types according to their effect on grassland: Type 1, those that kill or damage the grass; Type 2, those

which stimulate the grass only; Type 3, those having no effect on the grass but producing fructifications in rings. Type 1 rings have a well-defined mycelial zone in the soil, from which the fungus is usually easily isolated (Warcup, 1951b). They often show three rings in grass; an inner-zone where the grass is stimulated, a middle zone where the grass may be dead, and an outer zone where the grass is stimulated. Fructifications of the fungus are produced in the bare zone, or at the junction of the bare and outer zones, and may not be formed every year. The bare zone appears due to accumulation of mycelium in such large quantities that the soil becomes water-repellent (Shantz and Piemeisel, 1917; Bayliss Elliot, 1926; Warcup, 1951b) causing the grass to become "droughted out" under appropriate conditions. Mycelium of fairy rings grows outwards with a yearly increase of some 3–18 inches depending on the fungus and environmental conditions (Smith, 1957). Fairy rings are often visible on aerial photographs and large rings have been calculated to be several hundred years old (Ramsbottom, 1953). Warcup (1951b) showed that the mycelial zones of several Type 1 rings contain a restricted population of microfungi, both fewer species and fewer colonies as compared with uninvaded soil. Ascomycetes, including *Arachniotus*, *Chaetomium*, *Gymnoascus* and *Penicillium*, have been isolated more frequently from mycelial zones than from normal soil; it is possible that these fungi were present as resistant ascospores.

E. SPORULATION

1. Development of fructifications

Very little is known of the factors, internal or external, inducing reproduction in fungi, but it is probable that the conditions necessary for formation of the complex fructifications of Ascomycetes and Basidiomycetes are different from those for moulds. The principal factors involved in cessation of fungal growth are likely to be exhaustion of carbon or nitrogen supply, but undoubtedly many other factors are also concerned. Data show that in many cases growth may cease without sporulation, hyphae either "resting," forming hyphal segments or chlamydospores, or dying. Some fructifications, particularly oospores, are formed in plant tissue, others may occur in larger cavities in mineral soil, or on plant roots, or on plant or animal residues. Sporulation is common at the soil surface, on residues or in the litter layer.

Some fungi require light for sporulation; this varies from an absolute requirement for initiation or maturation of fructifications, to a quantitative response such as an increase in the number of sporophores upon illumination. Light is an absolute requirement for the formation of many different reproductive organs, including sporophores of Basidiomycetes, apothecia, perithecia, pycnidia, sporangia and conidia (Cochrane, 1958). On the other hand light is known to depress growth or sporulation of other fungi. The effect of light on sporulation of fungi occurring in soil has not been studied in any detail, but it is known that *Trichoderma viride* produces no or very few

conidia in continuous darkness, whereas in light it sporulates profusely (Barnett and Lilly, 1953; Miller and Reid, 1961), and that in *Fusarium* light brings about macrospore formation (Snyder and Hansen, 1941; Carlile, 1956; Reid, 1958).

Fructifications of larger fungi are often seasonal and many, though by no means all, fruit in autumn. According to Grainger (1946) and Wilkins and Harris (1946), autumn is the only season when soil temperature, moisture conditions and nitrogen supply are simultaneously adequate for formation of fruiting bodies. Hora (1959) has carried out some interesting experiments on the influence of various fertilizers on the number of Basidiomycete fructifications developing in Scots pine litter. He found that ammonium but not nitrate fertilizer increased sporophore number; lime decreased sporulation of most fungi but vastly increased the number of *Omphalia maura*, normally a very rare fungus in the area. In a few cases the requirements for fruiting have been investigated. Remsberg (1940) noted that sclerotia of *Typhula* normally germinated to give fructifications in wet cold weather in autumn and spring. She found that if sclerotia were refrigerated at 0–3° C with ultraviolet light for 2 hours daily they fruited in 2–4 weeks. Many members of the Sclerotiniaceae also require a rest period at 0° C before fructifications are obtained (Groves and Elliott, 1961). In a study of hypogeous fungi, Hawker (1954) noted the effect of contact stimuli by the frequency with which the fructifications of some species developed in contact with hard objects such as the edge of paths, clay hardpans, etc.

2. Spore dispersal

One of the surprising features of soil fungal studies, especially when using dilution plating techniques, is the wide dispersal of the population within a soil. Dobbs and Hinson (1960) and Burges (1960) have examined the distribution of fungal units in clods of soil. Small samples taken at 1 cm intervals on freshly exposed surfaces of clods were examined for their fungal populations. In each case the more common fungi were widely and relatively evenly distributed in the clods, and there appeared few "high counts" where sporulation might be considered to have taken place. Discussing their results, Dobbs and Hinson suggest that local accumulation of spores from sporulation must be very ephemeral, that spores seem to be subjected to a continual local mixing which gives every soil crumb a relatively uniform species composition. They consider that this mixing is carried out by members of the soil fauna. While soil dilution and soil plate data from Urrbrae loam mainly substantiate the results of Dobbs and Hinson, some of the less abundant fungi do occur in pockets where numbers are much larger than elsewhere. Similarly sclerotia, which are too large to be carried by most members of the soil fauna, often occur in areas of high or low density. Wensley and McKeen (1963) have found *Fusarium oxysporum* f. *melonis* in soil in loci of high or low concentration, the high loci being probably related to sites of infected plants in previous crops. Dick (1962) has also presented evidence that the distribution of the Saprolegniaceae within defined areas in soil is grouped and that the

distribution patterns thus recognizable are relatively constant over periods of many months at least.

Another factor of possible importance in mixing spores in soil is water movement. Hirst (1959) has commented that rain-scrubbing seems an ideal method of deposition for air-dispersed soil fungi. Burges (1950) studied the movement of spores through a sand column. He found that spores which had mucilaginous wettable coats may wash down readily, whereas spores with non-wetting coats remained at the surface. Dobbs and Hinson (1960) point out that with fungi from agar culture, wet spores pass through sand and dry spores are retained, but when a soil suspension is used no such differentiation occurs. Since Penicillia were numerous in the effluent, this would suggest that their conidia had lost their unwettable coat. Hepple (1960) studied the movement by water of spores through sand and certain horizons. She found that although such movement is possible under experimental conditions, its occurrence in the field is unlikely except over short distances. As Griffin (1963) points out, however, water movement of spores in natural soils is likely to occur where cracks, root channels, etc., are present. Movement and mixing, though not to great depths, also occurs when heavy rain breaks up soil clods in ploughed fields.

F. Destruction of Fungal Structures

The processes of fungal growth and sporulation in soil have been examined; the other side of the balance sheet, the ways in which fungal structures are degraded, must also be considered.

Lysis of young mycelia has been observed on many occasions (Chinn, 1953; Novogrudsky, 1948; Park, 1955; Stevenson, 1956; Tribe, 1957a; Saitô, 1960). Such lysis may be a result of purely internal metabolic changes (autolysis), as a result of contact with enzymes of other organisms, or as a result of exposure to toxic materials. Autolysis is usually a consequence of nutrient deficiency, but may also occur if utilization of energy sources is prevented by oxygen lack or through accumulation of by-products which are toxic to the organism producing them (Brian, 1960). Many toxic materials, including some antibiotics, will cause lysis.

Park (1961a) studied the growth in pure culture of 20 moulds, including Phycomycetes, Ascomycetes and Fungi Imperfecti. He found that in older cultures there was much hyphal lysis and all fungi had one or more types of resting structure in the cultures. Further study revealed that *Armillaria*, *Merulius* and *Xylaria*, fungi with bulky sporophores, did not show extensive lysis, had no resistant resting structures and maintained an intact hyphal network. *Pellicularia subcoronata*, a Basidiomycete without a complex sporophore, showed extensive lysis, however. This work shows that, at least in culture, autolysis is common in fungi.

1. Lysis

There is ample evidence that lysis may be caused by other organisms. Novogrudsky (1948) found that certain bacteria characteristically settled on

fungal hyphae, forming a bacterial sheath; lysis of the hyphae usually followed. Similar concentrations of bacteria around hyphae had been noted earlier (Cholodny, 1930; Conn, 1932). Starkey (1938) noted that while such bacterial-fungal associations were common, they were not seen with certain brown mycelium present in soil. Kovoor (1954) noted that severity of bactial attack on hyphae of *Rhizoctonia bataticola* depended on the moisture content of the soil and was much more severe at high moisture levels. Park (1956a) isolated a bacterium, *Bacillus macerans*, which would actively lyse fungi *in vitro*. Stevenson (1956) found that some *Streptomyces* could lyse *Helminthosporium sativum* in mixed culture in soil, and Lockwood (1959) recorded *Streptomyces* as a cause of natural fungitoxicity in soil. Mitchell and Alexander (1961a, b) found that the mycolytic bacteria, *Bacillus cereus*, *B. megaterium*, and a *Pseudomonas*, had high chitinase activity. They also found that addition of chitin to soil increased the number of chitin-decomposing organisms, particularly Actinomycetes, and markedly reduced the severity of root-rot of bean caused by *Fusarium solani* f. *phaseoli*. Further work showed that *B. cereus* and the *Pseudomonas* sp. digested living and dead *Fusarium* mycelium as well as cell wall preparations (Mitchell and Alexander, 1963).

Many fungal spores in soil germinate when residues are added, and evidence suggests that young germ tubes are particularly susceptible to lysis (Chinn and Ledingham, 1961). Brian (1960) considers that lysis under these conditions can probably be attributed to competition and antagonism by other organisms.

The question of antibiotics in soil has been extensively studied and direct evidence for their production has been difficult to obtain (Brian, 1957). Evidence for their production in localized environments such as fragments of plant material, seed coats or in the rhizosphere is gradually accumulating. Wright (1956a, b) buried wheat straws inoculated with *Trichoderma viride* in natural soil and after recovering them, she demonstrated the production of gliotoxin. Similar tests with viable spores of various fungi demonstrated the production of frequentin, gladiolic acid and gliotoxin. While specific antibiotics were not demonstrated, Witkamp (1960) found that powdered twigs naturally infected with *Trichoderma viride* added to agar markedly reduced the bacterial flora of soil-dilution plates. He also showed that mycelial strands of Basidiomycetes collected from oak litter inhibited *Bacillus subtilis* on agar media.

2. Parasitism

A number of soil fungi appear capable of parasitizing other fungi. Some are known parasites such as *Piptocephalis* and *Syncephalis* on members of the Mucorales; others belong to genera where parasitism would not be expected. Very little is known of the role of parasitic fungi on other fungi in soil. Boosalis (1956), in studies in natural soil, showed that *Penicillium vermiculatum* greatly reduced the viability of host hyphae of *Rhizoctonia solani*.

3. Predation

Members of the soil fauna feed on fungal structures in soil. In addition to nematodes and mites, thecamoebae, snails, slugs, some myriapods, Collembola, and certain beetles are all fungus eaters (Kuhnelt, 1961). The data of Tribe (1957, 1960a, b) give a good picture of some members of the soil fauna in relation to the fungi decomposing Cellophane film in soil. Sewell (1959c) working with plants in soil boxes, observed that *Verticillium* spore heads and distal parts of conidiophores were commonly eaten by Collembola and soil mites. Hooper (1962) found that *Aphelenchus avenae* almost completely destroyed mushroom mycelium in compost; this nematode occurs commonly in soil. Some preferences between mycelia of different fungi as food for soil animals have been noted. Winston (1956) found that few soil animals fed on the mycelium of *Armillaria mellea* compared with some other Basidiomycete mycelia. Jacot (1939) noted that *Lophodermium piceae* formed conspicuous black hyphal walls across fallen spruce needles through which feeding soil animals did not penetrate. Soil insects may affect fungi in further ways. Timonin (1961a, b) has shown that the volatile odoriferous material from the scent glands of the plant bug, *Scaptocoris talpa*, is fungistatic or fungicidal.

Witkamp (1960) has carried out a series of interesting experiments on the effects of members of the soil fauna on soil fungi. He estimated the amount of mycelium of *Mortierella pusilla* var. *isabellina* consumed by the springtail *Onychiurus armatus* and by 7 different species of Oribatid mites. Under favourable conditions one springtail consumed 6 m of hyphae a day; the consumption per day per mite was 0·8 m in summer and 0·2 in winter. Direct observation showed the presence of fungal spores adhering to the bristles of oribatid mites. Mites and springtails collected from oak litter and placed on agar transferred fungi which grew on the media; the fungi disseminated belonged predominantly to the genera *Penicillium*, *Mucor* and *Alternaria*. Slugs from fruiting bodies of Basidiomycetes also spread propagules of Fungi Imperfecti; similar observations were made by Talbot (1952). Each of a number of droppings of mites and springtails, sown on agar, gave rise to fungal colonies. On examination under the microscope, these droppings always appeared to contain fragments of mycelium, and in many cases spores were also observed. Poole (1957) showed that fungi may remain viable after passing through the gut of Collembola.

Adverse physical conditions may also kill fungi in soil. Drying causes death of both hyphae and spores (Warcup, 1957) and many fungal fructifications are very susceptible to drying. Flooding kills many, but not all, fungal structures in soil (Moore, 1949; Stover, 1953a).

V. CONCLUSIONS

Although it is possible to sketch outlines, it is evident that there are major gaps in our knowledge of the occurrence and growth of fungi in soil. New techniques will undoubtedly aid acquisition of fresh data, but it must be pointed out that there are many points of information which would greatly

aid our conception of fungal biology in soil which are capable of elucidation by present techniques. It is hoped that the time has come when a "species list" will not appear sufficient reward for study of organisms in soil; there are too many other interesting aspects waiting to be investigated.

REFERENCES

Ainsworth, G. C. (1961). "Ainsworth & Bisby's Dictionary of the Fungi." Commonwealth Mycological Institute, Kew, Surrey.

Ajello, L. (1956). *Science*, **123**, 876–879.

Alexander, F. E. S. and Jackson, R. M. (1955). *In* "Soil Zoology." (D. K. McE. Kevan, ed.), pp. 433–440. Butterworth, London.

Andersen, A. L. and Huber, D. (1962). *Phytopathology*, **52**, 1.

Anderson, E. J. (1951). *Phytopathology*, **41**, 187–189.

Apinis, A. E. (1958). *Angew. Pflsoziol.* **15**, 83–90.

Aytoun, R. S. C. (1953). *Trans. Proc. bot. Soc. Edinb.* **36**, 99–114.

Baker, K. F. (1953). *Plant Dis. Reptr.* **37**, 430–433.

Barnett, H. L. and Lilly, V. G. (1953). *Proc. W. Va. Acad. Sci.* **24**, 60–64.

Barton, R. (1957). *Nature, Lond.* **180**, 613–614.

Barton, R. (1958). *Trans. Br. mycol. Soc.* **41**, 207–222.

Bayliss, Elliott, J. S. (1926). *Ann. appl. Biol.* **13**, 277–288.

Bayliss Elliott, J. S. (1930). *Ann. appl. Biol.* **17**, 284–305.

Beckwith, T. D. (1911). *Phytopathology*, **1**, 169–176.

Bisby, G. R., James, N. and Timonin, M. I. (1933). *Can. J. Res. C*, **8**, 253–275.

Bisby, G. R., Timonin, M. I. and James, N. (1935). *Can. J. Res. C*, **13**, 47–65.

Blackwell, E. (1943). *Trans. Br. mycol. Soc.* **26**, 71–89.

Blair, I. D. (1945). *N.Z. J. Sci. Technol.* (Sec. A) **26**, 258–271.

Bolliger, A. and Gross, R. (1952). *Aust. J. exp. Biol. med. Sci.* **30**, 399–408.

Boosalis, M. G. (1956). *Phytopathology*, **46**, 473–478.

Boosalis, M. G. and Scharen, A. L. (1959). *Phytopathology*, **49**, 192–198.

Boswell, J. G. and Sheldon, J. (1951). *New Phytol.* **50**, 172–178.

Bracker, C. E. and Butler, E. E. (1963). *Mycologia*, **55**, 35–58.

Brian, P. W. (1957). *In 7th Symp. Soc. gen. Microbiol.* pp. 168–188. Cambridge University Press, London.

Brian, P. W. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 115–129. Liverpool University Press.

Brierley, W. B. (1923). *In* "The Micro-organisms of the Soil." (Sir John Russell, ed.), pp. 118–146. Longmans, Green and Co., London.

Brierley, W. B., Jewson, S. T. and Brierley, M. (1927). *1st Int. Congr. Soil Sci.* **3**, 48–71.

Brown, J. C. (1958a). *Trans. Br. mycol. Soc.* **41**, 81–88.

Brown, J. C. (1958b). *J. Ecol.* **46**, 641–664.

Burges, A. (1950). *Trans. Br. mycol. Soc.* **33**, 142–147.

Burges, A. (1958). "Micro-organisms in the Soil." Hutchinson, London.

Burges, A. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 185–191. Liverpool University Press.

Burges, A. (1963). *Trans. Br. mycol. Soc.* **46**, 1–14.

Burges, A. and Fenton, E. (1953). *Trans. Br. mycol. Soc.* **36**, 104–108.

Burges, A. and Nicholas, D. P. (1961). *Soil Sci.* **92**, 25–29.

Butler, E. E. (1957). *Mycologia*, **49**, 354–373.

Butler, E. J. (1939). *Trans. Br. mycol. Soc.* **22**, 274–301.
Butler, F. C. (1953). *Ann. appl. Biol.* **40**, 284–311.
Buxton, E. W. and Kendrick, J. B. (1963). *Ann. appl. Biol.* **51**, 215–221.
Caldwell, R. (1958). *Nature, Lond.* **181**, 1144–1145.
Campbell, W. A. (1947). *Mycologia*, **39**, 190–195.
Campbell, W. A. (1949). *Plant. Dis. Reptr.* **33**, 134–135.
Campbell, W. P. (1956). *Can. J. Bot.* **34**, 865–874.
Capstick, C. K., Twinn, D. C. and Waid, J. S. (1957). *Nematologica*, **2**, 193–201.
Carlile, M. J. (1956). *J. gen. Microbiol.* **14**, 643–654.
Carter, M. V. and Moller, W. J. (1961). *Aust. J. agric. Res.* **12**, 878–888.
Chesters, C. G. C. (1940). *Trans. Br. mycol. Soc.* **24**, 352–355.
Chesters, C. G. C. (1948). *Trans. Br. mycol. Soc.* **30**, 100–117.
Chesters, C. G. C. (1949). *Trans. Br. mycol. Soc.* **32**, 197–216.
Chesters, C. G. C. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 223–238. Liverpool University Press.
Chesters, C. G. C. and Thornton, R. H. (1956). *Trans. Br. mycol. Soc.* **39**, 301–313.
Chinn, S. H. F. (1953). *Can. J. Bot.* **31**, 718–724.
Chinn, S. H. F. and Ledingham, R. J. (1957). *Can. J. Bot.* **35**, 697–701.
Chinn, S. H. F. and Ledingham, R. J. (1958). *Can. J. Bot.* **36**, 289–295.
Chinn, S. H. F. and Ledingham, R. J. (1961). *Can. J. Bot.* **39**, 739–748.
Chinn, S. H. F., Ledingham, R. J., Sallans, B. J. and Simmonds, P. M. (1953). *Phytopathology*, **43**, 701.
Cholodny, N. (1930). *Arch. Mikrobiol.* **1**, 620–652.
Christensen, M., Whittingham, W. F. and Novak, R. O. (1962). *Mycologia*, **54**, 374–388.
Christou, T. (1962a). *Phytopathology*, **52**, 194–198.
Christou, T. (1962b). *Phytopathology*, **52**, 381–389.
Christou, T. and Snyder, W. C. (1962). *Phytopathology*, **52**, 219–226.
Clarke, J. H. and Parkinson, D. (1960). *Nature, Lond.* **188**, 166–167.
Cochrane, V. W. (1958). "Physiology of Fungi." John Wiley and Sons, New York.
Cochrane, V. W. (1960). *In* "Plant Pathology." (J. G. Horsfall and A. E. Dimond, eds.), vol. II, pp. 169–202. Academic Press, New York.
Coley-Smith, J. R. (1959). *Ann. appl. Biol.* **47**, 511–518.
Coley-Smith, J. R. (1960). *Ann. appl. Biol.* **48**, 8–18.
Conn, H. J. (1918). *Tech. Bull. N.Y. St. agric. Exp. Stn*, **64**, 1–20.
Conn, H. J. (1932). *Tech. Bull. N.Y. St. agric. Exp. Stn*, **204**, 3–31.
Cooke, R. C. (1962). *Trans. Br. mycol. Soc.* **45**, 314–320.
Cooke, W. B. (1958). *Bot. Rev.* **24**, 341–429.
Cooke, W. B. and Lawrence, D. B. (1959). *J. Ecol.* **47**, 529–549.
Corner, E. J. H. (1950). *Ann. Bot. Mem. No.* 1, Oxford.
Cowley, G. T. and Whittingham, W. F. (1961). *Mycologia*, **53**, 539–542.
Cunningham, J. L. and Hagedorn, D. J. (1962). *Phytopathology*, **52**, 616–618.
Dale, E. (1912). *Ann. Mycol.* **10**, 452–477.
Dale, E. (1914). *Ann. Mycol.* **12**, 33–62.
Daniels, J. (1961). *Trans. Br. mycol. Soc.* **44**, 79–86.
Dawson, V. T. and Dawson, R. C. (1946). *Proc. Soil Sci. Am.* **11**, 268–269.
Dean, A. L. (1929). *Phytopathology*, **19**, 407–412.
Demeter, K. J. and Mossel, H. (1933). *Zbl. Bakt.* (11) **88**, 384–393.
Vries, G. A. de. (1962). *Antonie van Leeuwenhoek*, **28**, 121–133.
Dick, M. W. (1962). *J. Ecol.* **50**, 119–127.

Dobbs, C. G. and Carter, N. C. C. (1963). Report on Forest Research for 1962, pp. 103–112. H.M.S.O. London.
Dobbs, C. G. and Hinson, W. H. (1953). *Nature, Lond.* **172**, 197–199.
Dobbs, C. G. and Hinson, W. H. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 33–42. Liverpool University Press.
Dobbs, C. G., Hinson, W. H. and Bywater, J. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 130–147. Liverpool University Press.
Drechsler, C. (1941). *Biol. Rev.* **16**, 265–290.
Duddington, C. L. (1955). *Bot. Rev.* **21**, 377–439.
Duddington, C. L. (1957). "The Friendly Fungi." Faber and Faber, London.
Dulaney, E. L., Larsen, A. H. and Stapley, E. O. (1955). *Mycologia*, **47**, 420–422.
Durbin, R. D. (1959). *Am. J. Bot.* **46**, 22–25.
Durbin, R. D. (1961). *Bot. Rev.* **27**, 522–560.
Durbin, R. D., Davis, L. H. and Baker, K. F. (1955). *Phytopathology*, **45**, 509–512.
Durrell, L. W. and Shields, L. M. (1960). *Mycologia*, **52**, 636–641.
Eastwood, D. J. (1952). *Trans. Br. mycol. Soc.* **35**, 215–220.
Eaton, E. D. and King, C. J. (1934). *J. agric. Res.* **49**, 1109–1113.
Eckert, J. W. and Tsao, P. H. (1962). *Phytopathology*, **52**, 771–777.
Ellis, J. J. and Hesseltine, C. W. (1962). *Nature, Lond.* **193**, 699–700.
Ellis, M. (1940). *Trans. Br. mycol. Soc.* **24**, 87–97.
Emmons, C. W. (1951). *J. Bact.* **62**, 685–690.
Ezekiel, W. N. (1940). *Rep. Tex. agric. Exp. Stn*, **1939**, 84–86.
Ezekiel, W. N. (1945). *Phytopathology*, **35**, 296–301.
Farrow, W. M. (1954). *Mycologia*, **46**, 632–646.
Fennell, D. I. (1960). *Bot. Rev.* **26**, 79–141.
Ferguson, J. (1953). *Phytopathology*, **43**, 471.
Gadd, C. H. and Bertus, L. S. (1928). *Ann. R. bot. Gdns Peradeniya*, **11**, 27–49.
Gams, W. (1959). *Sydowia*, **13**, 87–94.
Garrett, S. D. (1937). *Ann. appl. Biol.* **24**, 747–751.
Garrett, S. D. (1944). "Root Disease Fungi." Chronica Botanica, Waltham, Mass., U.S.A. (*Annales Cryptogamici et Phytopathologici*, Vol. 1).
Garrett, S. D. (1944). *Chronica bot.*
Garrett, S. D. (1951). *New Phytol.* **50**, 149–166.
Garrett, S. D. (1952). *Sci. Progr. Lond.* **159**, 436–450.
Garrett, S. D. (1954). *Trans. Br. mycol. Soc.* **37**, 51–57.
Garrett, S. D. (1955). *Trans. Br. mycol. Soc.* **38**, 1–9.
Garrett, S. D. (1956). "Biology of Root-infecting Fungi." Cambridge University Press, London.
Gerdemann, J. W. (1955). *Mycologia*, **47**, 619–632.
Gerdemann, J. W. and Nicolson, T. H. (1963). *Trans. Br. mycol. Soc.* **46**, 235–244.
Gilman, J. C. (1957). "A Manual of Soil Fungi." Iowa State College Press, Ames, Iowa.
Grainger, J. (1946). *Trans. Br. mycol. Soc.* **29**, 52–63.
Grainger, J. (1962). *Trans. Br. mycol. Soc.* **45**, 147–155.
Greenwood, D. J. (1961). *Pl. Soil*, **14**, 360–376.
Griffin, D. M. (1960). *Trans. Br. mycol. Soc.* **43**, 583–596.
Griffin, D. M. (1963). *Biol. Rev.* **38**, 141–166.
Griffin, G. J. (1962). *Phytopathology*, **52**, 90–91.
Griffiths, E. and Jones, D. (1963). *Trans. Br. mycol. Soc.* **46**, 285–294.

Griffiths, E. and Siddiqi, M. A. (1961). *Trans. Br. mycol. Soc.* **44**, 343–353.
Groves, J. W. and Elliott, M. E. (1961). *Can. J. Bot.* **39**, 215–231.
Guillemat, J. and Montégut, J. (1957). *Annls. Épiphyt.* **8**, 185–207.
Haarløv, N. and Weis-Fogh, T. (1953). *Oikos*, **4**, 44–57.
Haarløv, N. and Weis-Fogh, T. (1955). *In* "Soil Zoology." (D. K. McE. Kevan, ed.), pp. 429–432. Butterworth, London.
Hagem, O. (1907). *Math. naturw. Klasse Bd*, **7**, 1–50.
Hagem, O. (1910). *Annls. mycol.* **8**, 265–286.
Harley, J. L. (1959). "The Biology of Mycorrhiza." Leonard Hill, London.
Harley, J. L. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 265–276. Liverpool University Press.
Harley, J. L. and Waid, J. S. (1955). *Trans. Br. mycol. Soc.* **38**, 104–118.
Harvey, J. V. (1925). *J. Elisha Mitchell scient. Soc.* **41**, 151–164.
Hawker, L. E. (1954). *Phil. Trans. R. Soc. B*, **237**, 429–546.
Hawker, L. E. (1955a). *Biol. Rev.* **30**, 127–158.
Hawker, L. E. (1955b). *Trans. Br. mycol. Soc.* **38**, 73–77.
Hawker, L. E. (1957a). "The Physiology of Reproduction in Fungi." Cambridge University Press, London.
Hawker, L. E. (1957b). *In* "Microbial Ecology." *7th Symp. Soc. gen. Microbiol.*, pp. 238–258. Cambridge University Press, London.
Hepple, S. (1958). *Mucor ramannianus* in a podsolized soil. Ph.D. thesis, University of Liverpool.
Hepple, S. (1960). *Trans. Br. mycol. Soc.* **43**, 73–79.
Hepple, S. and Burges, A. (1956). *Nature, Lond.* **177**, 1186.
Hessayon, D. G. (1953). *Soil Sci.* **75**, 395–404.
Hesselman, H. (1926). *Medd. Skogsförsöksanst Stockh.* **22**, 169–552.
Hickman, C. J. (1958). *Trans. Br. mycol. Soc.* **41**, 1–13.
Hildebrand, A. A. and Koch, L. W. (1936). *Can. J. Res. C*, **14**, 11–26.
Hine, R. B. (1962). *Phytopathology*, **52**, 736.
Hirst, J. M. (1959). *In* "Plant Pathology: Problems and Progress 1908–1958." (C. S. Holton, ed.), pp. 529–538. University of Wisconsin Press, Madison.
Hooper, D. J. (1962). *Nature, Lond.* **193**, 496–497.
Hora, F. B. (1959). *Trans. Br. mycol. Soc.* **42**, 1–14.
How, J. E. (1940). *Ann. Bot., N.S.* **4**, 135–150.
Jackson, R. M. (1957). *Nature, Lond.* **180**, 96–97.
Jackson, R. M. (1958a). *J. gen. Microbiol.* **18**, 248–258.
Jackson, R. M. (1958b). *J. gen. Microbiol.* **19**, 390–401..
Jackson, R. M. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 168–176. Liverpool University Press.
Jacot, A. P. (1939). *J. For.* **37**, 858–860.
James, N. (1959). *Can. J. Microbiol.* **5**, 431–439.
James, N. and Sutherland, M. L. (1939). *Can. J. Res. C*, **17**, 97–108.
Jefferys, E. G. and Hemming, H. G. (1953). *Nature, Lond.* **172**, 872–873.
Jensen, C. N. (1912). *Bull. N.Y. (Cornell) agric. Exp. Stn*, **315**, 415–501.
Jensen, H. L. (1931). *Soil Sci.* **31**, 123–158.
Jensen, H. L. (1934). *Proc. Linn. Soc. N.S.W.* **59**, 200–211.
Jensen, H. L. (1935). *Proc. Linn. Soc. N.S.W.* **60**, 145–154.
Joffe, A. Z. (1963). *Mycologia*, **55**, 271–282.
Johnson, L. F. and Manka, K. (1961). *Soil Sci.* **92**, 79–84.
Jones, P. C. T. and Mollinson, J. E. (1948). *J. gen. Microbiol.* **2**, 54–69.

Kendrick, W. B. (1958). *Nature, Lond.* **181**, 432.
Kendrick, W. B. (1959). *Can. J. Bot.* **37**, 907–912.
Kendrick, W. B. and Burges, A. (1962). *Nova Hedwigia*, **4**, 313–342.
Kerr, A. (1963). *Aust. J. biol. Sci.* **16**, 55–69.
Keynan, A., Henis, Y. and Keller, P. (1961). *Nature, Lond.* **191**, 307.
Keyworth, W. G. (1951). *Trans. Br. mycol. Soc.* **34**, 291–292.
King, C. J., Loomis, H. F. and Hope, C. (1931). *J. agric. Res.* **42**, 827–840.
Klotz, L. J. and Fawcett, H. S. (1939). *Phytopathology*, **29**, 290–291.
Kovoor, A. T. A. (1954). *J. Madras Univ.* B, **24**, 47–52.
Krzemieniewska, H. and Badura, L. (1954). *Acta Soc. Bot. Polon.* **23**, 727–781.
Kubiena, W. L. (1938). "Micropedology." Collegiate Press, Inc., Iowa.
Kubíková, J. (1963). *Trans. Br. mycol. Soc.* **46**, 107–114.
Kurbis, W. P. (1937). *Flora, Jena*, **131**, 129–175.
Kuhnelt, W. (1961). "Soil Biology." Faber and Faber, London.
La Touche, C. J. (1948). *Trans. Br. mycol. Soc.* **31**, 281–284.
Lebeau, J. B. and Cormack, M. W. (1956). *Phytopathology*, **46**, 298.
Lebeau, J. B. and Logsdon, C. E. (1958). *Phytopathology*, **48**, 148–150.
Ledingham, R. J. and Chinn, S. H. F. (1955). *Can. J. Bot.* **33**, 298–303.
Legge, B. J. (1952). *Nature, Lond.* **169**, 759–760.
Lendner, A. (1908). *Beitr. Kryptogflora Schweiz*, **3**, 1–180.
Levisohn, I. (1955). *Nature, Lond.* **176**, 519.
Linford, M. B. (1942). *Soil Sci.* **53**, 93–103.
Lingappa, B. T. and Lockwood, J. L. *J. gen. Microbiol.* **26**, 473–485.
Lingappa, B. T. and Lockwood, J. L. (1962). *Phytopathology*, **52**, 295–299.
Lingappa, B. T. and Lockwood, J. L. (1963). *Phytopathology*, **53**, 529–531.
Lingappa, Y. and Lockwood, J. L. (1961). *Nature, Lond.* **189**, 158–159.
Lloyd, A. B. and Lockwood, J. L. (1962). *Phytopathology*, **52**, 1314–1315.
Lockwood, J. L. (1959). *Phytopathology*, **49**, 327–331.
Lockwood, J. L. and Lingappa, B. T. (1963). *Phytopathology*, **53**, 917–920.
Lýsek, H. (1963). *Nature, Lond.* **199**, 925.
McDonough, E. S., Ajello, L., Austermann, R. J., Balows, A., McClellan, J. T. and Brinkman, S. (1961). *Am. J. Hyg.* **73**, 75–83.
MacFarlane, I. (1952). *Ann. appl. Biol.* **39**, 239–256.
McKeen, W. E. (1952). *Can. J. Bot.* **30**, 344–347.
McLennan, E. (1928). *Ann. appl. Biol.* **15**, 95–109.
McLennan, E. I. and Ducker, S. C. (1954). *Aust. J. Bot.* **2**, 220–245.
McTeague, D. M., Hutchinson, S. A. and Reed, R. I. (1959). *Nature, Lond.* **183**, 1736.
MacWithey, H. S. (1957). *Rept 36th A. Conv. NWest. Ass. Hortic., Entomols Pl. Pathol.* pp. 5–6.
Maloy, O. C., and Alexander, M. (1958). *Phytopathology*, **48**, 126–128.
Martin, J. P. (1950). *Soil Sci.* **69**, 215–232.
Martin, T. L., Anderson, D. A. and Goates, R. (1942). *Soil Sci.* **54**, 297–302.
Martinson, C. and Baker, R. (1962). *Phytopathology*, **52**, 619–621.
Melin, E. (1925). "Untersuchungen über die Bedeutung der Baummykorrhiza," pp. 1–152. G. Fischer, Jena.
Melin, E. (1946). *Symb. bot. Upsal.* **8**, 1–116.
Melin, E. (1954). *Svensk bot. Tidskr.* **48**, 86–94.
Melin, E. and Das, V. S. R. (1954). *Physiologia Pl.* **7**, 851–858.
Mikola, P. (1948). *Commn. Inst. For. Fenn.* **36**, 1–104.

Mikola, P. (1956). *Commn. Inst. For. Fenn.* **48**, 5–22.
Miller, P. M. (1956). *Phytopathology*, **46**, 526.
Miller, J. H., Giddens, J. E. and Foster, A. A. (1957). *Mycologia*, **49**, 779–808.
Miller, J. J. and Reid, J. (1961). *Can. J. Bot.* **39**, 259–262.
Miller, J. J. and Webb, N. S. (1954). *Soil Sci.* **77**, 197–204.
Minderman, G. (1956). *Pl. Soil* **8**, 42–48.
Mitchell, R. B., Hooton, D. R. and Clark, F. E. (1941). *J. agric. Res.* **63**, 535–547.
Mitchell, R. and Alexander, M. (1961a). *Nature, Lond.* **190**, 109–110.
Mitchell, R. and Alexander, M. (1961b). *Plant Dis. Reptr.* **45**, 487–490.
Mitchell, R. and Alexander, M. (1963). *Can. J. Microbiol.* **9**, 169–177.
Molin, N. (1957). *Medd. Skogsförsöksanst. Stockh.* **47**, 1–36.
Montégut, J. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 43–52. Liverpool University Press.
Moore, J. J. (1954). *Scient. Proc. R. Dubl. Soc.* **26**, 379–395.
Moore, W. D. (1949). *Phytopathology*, **39**, 920–927.
Morrow, M. B. (1932). *Mycologia*, **24**, 398–402.
Mosse, B. (1959). *Trans. Br. mycol. Soc.* **42**, 439–448.
Mueller, K. E. and Durrell, L. W. (1957). *Phytopathology*, **47**, 243.
Muskett, A. E. (1960). *In* "Plant Pathology." (J. G. Horsfall and A. E. Dimond, eds.), vol. III, pp. 58–96. Academic Press, New York.
Nash, S. M. and Snyder, W. C. (1962). *Phytopathology*, **52**, 567–572.
Nash, S. M., Christou, T. and Snyder, W. C. (1961). *Phytopathology*, **51**, 308–312.
Newcombe, M. (1960). *Trans. Br. mycol. Soc.* **43**, 51–59.
Newhook, F. J. (1959). *N.Z. Jl. agric. Res.* **2**, 808–843.
Nicolson, T. H. (1959). *Trans. Br. mycol. Soc.* **42**, 421–438.
Nicot, J. (1953). *Rev. Mycol.* **18** (*suppl. colon* 2), 88–93.
Nicot, J. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 94–97. Liverpool University Press.
Nicot, J. and Chevaugeon, J. (1949). *Bull. Mus. Hist. nat. Paris* **31**, 384–392.
Norkrans, B. (1949). *Svensk bot. Tidskr.* **43**, 485–490.
Novogrudsky, D. M. (1948). *Mikrobiologiya*, **17**, 28–35.
Ohms, R. E. (1957). *Phytopathology*, **47**, 751–752.
Orpurt, P. A. and Curtis, J. T. (1957). *Ecology*, **38**, 628–637.
Oudemans, C. A. J. A. and Koning, C. J. (1902). *Archs. néerl. Sci. Série 2*, **7**, 286–298.
Pady, S. M., Kramer, C. L. and Pathak, V. K. (1960). *Mycologia*, **52**, 347–350.
Paharia, K. D. and Kommedahl, T. (1956). *Plant. Dis. Reptr.* **40**, 1029–1031.
Pantidou, M. E. (1961). *Can. J. Bot.* **39**, 1149–1162.
Papavizas, G. C. and Davey, C. B. (1959a). *Plant. Dis. Reptr.* **43**, 404–410.
Papavizas, G. C. and Davey, C. B. (1959b). *Soil Sci.* **88**, 112–117.
Papavizas, G. C. and Davey, C. B. (1961a). *Phytopathology*, **51**, 92–96.
Papavizas, G. C. and Davey, C. B. (1961b). *Phytopathology*, **61**, 693–699.
Papavizas, G. C. and Davey, C. B. (1961c). *Pl. Soil* **14**, 215–236.
Papavizas, G. C. and Davey, C. B. (1962). *Phytopathology*, **52**, 834–840.
Park, D. (1954). *Nature, Lond.* **173**, 454–455.
Park, D. (1955). *Trans. Br. mycol. Soc.* **38**, 130–142.
Park, D. (1956a). *Trans. Br. mycol. Soc.* **39**, 239–259.
Park, D. (1956b). *6th Int. Congr. Soil Sci.* **3**, 23–28.
Park, D. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 148–159. Liverpool University Press.

Park, D. (1961a). *Trans. Br. mycol. Soc.* **44**, 119–122.
Park, D. (1961b). *Trans. Br. mycol. Soc.* **44**, 377–390.
Parkinson, D. (1957). *Pedologie*, **7**, *no. spéc.* (*Symp. Meth. Et. Microbiol. Sol*), 146–154.
Parkinson, D. and Chesters, C. G. C. (1958). *Nature, Lond.* **181**, 1746–1747.
Parkinson, D. and Clarke, J. H. (1961). *Pl. Soil*, **13**, 384–390.
Parkinson, D. and Kendrick, W. B. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 22–28. Liverpool University Press.
Parkinson, D. and Williams, S. T. (1961). *Pl. Soil*, **13**, 347–355.
Parmeter, J. R. and Hood, J. R. (1961). *Phytopathology*, **51**, 164–168.
Peltier, G. L. (1937). *Phytopathology*, **27**, 145–158.
Peterson, E. A. (1958). *Can. J. Microbiol.* **4**, 257–265.
Peyronel, B. (1924). *Boll. Staz. Patol. veg. Roma*, **5**, 73–75.
Poole, T. B. (1957). Report on Forest Research for 1956, pp. 109–111. H.M.S.O. London.
Pugh, G. J. F. (1958). *Trans. Br. mycol. Soc.* **41**, 185–195.
Pugh, G. J. F. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 202–208. Liverpool University Press.
Pugh, G. J. F. and Mathison, G. E. (1962). *Trans. Br. mycol. Soc.* **45**, 567–572.
Ramsbottom, J. (1953). "Mushrooms and Toadstools." Collins, London.
Rayss, T. and Borut, S. (1958). *Mycopath. Mycol. appl.* **10**, 142–174.
Reid, J. (1958). *Can. J. Bot.* **36**, 507–537.
Remsburg, R. E. (1940). *Mycologia*, **32**, 52–96.
Rishbeth, J. (1950). *Ann. Bot. N.S.* **14**, 365–383.
Robertson, N. F. (1954). *New Phytol.* **53**, 253–283.
Roistacher, C. N., Baker, K. F. and Bald, J. G. (1957). *Hilgardia*, **26**, 659–684.
Romell, L. G. (1935). *Mem. Cornell Univ. agric. Exp. Stn*, **170**, 1–28.
Rossi, G. M. (1928). *Italia agric.* **4**.
Russell, E. W. (1961). "Soil Conditions and Plant Growth." (9th Edition) Longmans, Green and Co. London.
Russell, P. (1956). *Nature, Lond.* **177**, 1038–1039.
Sadasivan, T. S. (1939). *Ann. appl. Biol.* **26**, 497–508.
Saitô, T. (1952). *Ecol. Rev. Japan*, **13**, 111–119.
Saitô, T. (1955a). *Ecol. Rev. Japan*, **14**, 69–74.
Saitô, T. (1955b). *Sci. Rep. Tôhoku Univ. 4th Ser.* **21**, 145–151.
Saitô, T. (1956). *Ecol. Rev. Japan*, **14**, 141–147.
Saitô, T. (1957). *Ecol. Rev. Japan*, **14**, 209–216.
Saitô, T. (1958). *Sci. Rep. Tôhoku Univ. 4th Ser.* **24**, 73–79.
Saitô, T. (1960). *Sci. Rep. Tôhoku Univ. 4th Ser.* **26**, 125–131.
Scharen, A. L. (1960). *Phytopathology*, **50**, 274–277.
Schmitthenner, A. F. (1962). *Phytopathology*, **52**, 1133–1138.
Schroth, M. N. and Hendrix, F. F. (1962). *Phytopathology*, **52**, 906–909.
Schroth, M. N. and Snyder, W. C. (1961). *Phytopathology*, **51**, 389–393.
Schütte, K. H. (1956). *New Phytol.* **55**, 164–182.
Scott, A. D. and Evans, D. D. (1955). *Proc. Soil Sci. Soc. Am.* **19**, 7–12.
Sewell, G. W. F. (1956). *Nature, Lond.* **177**, 708.
Sewell, G. W. F. (1959a). *Trans. Br. mycol. Soc.* **42**, 343–353.
Sewell, G. W. F. (1959b). *Trans. Br. mycol. Soc.* **42**, 354–369.
Sewell, G. W. F. (1959c). *Trans. Br. mycol. Soc.* **42**, 312–321.

Sewell, G. W. F. (1959d). *New. Phytol.* **58**, 5–15.
Shantz, H. L. and Piemeisel, R. L. (1917). *J. agric. Res.* **11**, 191–245.
Simmonds, P. M. (1930). *Phytopathology*, **20**, 911–913.
Simmonds, P. M. and Ledingham, R. J. (1937). *Scient. Agric.* **18**, 49–59.
Singh, R. S. and Mitchell, J. E. (1961). *Phytopathology*, **51**, 440–444.
Smith, J. D. (1957). *J. Sports Turf Res. Inst.* **9**, 324–352.
Smith, N. R. and Dawson, V. T. (1944). *Soil Sci.* **58**, 467–471.
Snyder, W. C. and Hansen, H. N. (1941). *Mycologia*, **33**, 580–591.
Sparrow, F. K. (1957). *Trans. Br. mycol. Soc.* **40**, 523–535.
Stanier, R. Y. (1953). *In* "Adaptation in Micro-organisms." *3rd Symp. Soc. gen. Microbiol.* Cambridge University Press, London.
Starkey, R. L. (1929). *Soil Sci.* **27**, 319–334.
Starkey, R. L. (1938). *Soil Sci.* **45**, 207–249.
Stenton, H. (1953). *Trans. Br. mycol. Soc.* **36**, 304–314.
Stenton, H. (1958). *Trans. Br. mycol. Soc.* **41**, 74–80.
Stevenson, I. L. (1956). *J. gen. Microbiol.* **15**, 372–380.
Stover, R. H. (1953a). *Can. J. Bot.* **31**, 693–697.
Stover, R. H. (1953b). *Nature, Lond.* **172**, 465.
Stover, R. H. (1958). *Can. J. Bot.* **36**, 439–453.
Swart, H. J. (1958). *Acta bot. neerland.* **7**, 741–768.
Talbot, P. H. B. (1952). *Trans. Br. mycol. Soc.* **35**, 123–128.
Taubenhaus, J. J. and Ezekiel, W. N. (1930). *Phytopathology*, **20**, 761–785.
Thornton, R. H. (1952). *Research, Lond.* **5**, 190–191.
Thornton, R. H. (1956a). *Trans. Br. mycol. Soc.* **39**, 485–494.
Thornton, R. H. (1956b). *Nature, Lond.* **177**, 230–231.
Thornton, R. H. (1958). *Nature, Lond.* **182**, 1690.
Thornton, R. H. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 84–91. Liverpool University Press.
Thornton, R. H., Cowie, J. D. and McDonald, D. C. (1956). *Nature, Lond.* **177**, 231–232.
Tichelaar, G. M. (1961). *Tijdschr. PlZiekt.* **67**, 290–295.
Timonin, M. I. (1961a). *Pl. Soil*, **14**, 323–334.
Timonin, M. I. (1961b). *Can. J. Bot.* **39**, 695–703.
Toussoun, T. A., Nash, S. M. and Snyder, W. C. *Phytopathology*, **50**, 137–140.
Toussoun, T. A. and Patrick, Z. A. (1962). *Phytopathology*, **52**, 30.
Toussoun, T. A. and Snyder, W. C. (1961). *Phytopathology*, **51**, 620–623.
Tracey, M. V. (1956). *Rep. Rothamsted exp. Stn.* 1955, pp. 87–88.
Tresner, H. D., Backus, M. P. and Curtis, J. T. (1954). *Mycologia*, **46**, 314–333.
Tribe, H. T. (1957a). *7th Symp. Soc. gen. Microbiol.* 287–298. Cambridge University Press, London.
Tribe, H. T. (1957b). *Trans. Br. mycol. Soc.* **40**, 489–499.
Tribe, H. T. (1960a). *Can. J. Microbiol.* **6**, 309–316.
Tribe, H. T. (1960b). *Can. J. Microbiol.* **6**, 317–323.
Tribe, H. T. (1960c). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 246–256. Liverpool University Press.
Tribe, H. T. (1961). *Soil Sci.* **92**, 61–77.
Triffitt, M. J. (1935). *J. Helminth.* **13**, 59–66.
Vaartaja, O. (1960). *Phytopathology*, **50**, 870–873.
Vanbreuseghem, R. (1952). *Annls, Soc. belge Méd. trop.* **32**, 173–178.
van der Laan, P. A. (1956). *Tijdschr. PlZiekt.* **62**, 305–321.

Vanterpool, T. C. and Macrae, R. (1951). *Can. J. Bot.* **29**, 147–157.
Veldkamp, H. (1955). *Meded. LandbouwHoogesch. Wageningen*, **55**, 127–174.
Venkat Ram, C. S. (1952). *Nature, Lond.* **170**, 889.
Waid, J. S. (1957). *Trans. Br. mycol. Soc.* **40**, 391–406.
Waid, J. S. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 55–75. Liverpool University Press.
Waid, J. S. and Woodman, M. J. (1957). *Pedologie*, **7**, no. spéc. (*Symp. Méth. Et. Microbiol. Sol*), pp. 155–158.
Waksman, S. A. (1916a). *Science*, N.S. **44**, 320–322.
Waksman, S. A. (1916b). *Soil Sci.* **2**, 103–155.
Waksman, S. A. (1917). *Soil Sci.* **3**, 565–589.
Waksman, S. A. (1922). *J. Bact.* **7**, 339–341.
Waksman, S. A. (1927). "Principles of Soil Microbiology." Baillière, Tindall and Cox, London.
Waksman, S. A. (1944). *Soil Sci.* **58**, 89–114.
Wallace, H. A. H. (1959). *Can. J. Bot.* **37**, 509–515.
Warcup, J. H. (1950) *Nature, Lond.* **166**, 117.
Warcup, J. H. (1951a). *Trans. Br. mycol. Soc.* **34**, 376–399.
Warcup, J. H. (1951b). *Ann. Bot. N.S.* **15**, 305–317.
Warcup, J. H. (1952). *Trans. Br. mycol. Soc.* **35**, 248–262.
Warcup, J. H. (1955a). *Nature, Lond.* **175**, 953.
Warcup, J. H. (1955b). *Trans. Br. mycol. Soc.* **38**, 298–301.
Warcup, J. H. (1957). *Trans. Br. mycol. Soc.* **40**, 237–262.
Warcup, J. H. (1959). *Trans. Br. mycol. Soc.* **42**, 45–52.
Warcup, J. H. (1960). *In* "The Ecology of Soil Fungi." (D. Parkinson and J. S. Waid, eds.), pp. 3–21. Liverpool University Press.
Warcup, J. H. and Baker, K. F. (1963). *Nature, Lond.* **197**, 1317–1318.
Warcup, J. H. and Talbot, P. H. B. (1962). *Trans. Br. mycol. Soc.* **45**, 495–518.
Warcup, J. H. and Talbot, P. H. B. (1963). *Trans. Br. mycol. Soc.* **46** (4).
Warren, J. R. (1948). *Mycologia*, **40**, 391–401.
Watling, R. (1963). *Trans. Br. mycol. Soc.* **46**, 81–90.
Weber, G. F. (1929). *Mycologia*, **21**, 113–130.
Webley, D. M., Eastwood, D. J. and Gimingham, C. H. (1952). *J. Ecol.* **40**, 168–178.
Weindling, R. (1932). *Phytopathology*, **22**, 837–845.
Weinhold, A. R. and Hendrix, F. F. (1962). *Phytopathology*, **52**, 32.
Wensley, R. N. and McKeen, C. D. (1963). *Can. J. Microbiol.* **9**, 237–249.
Went, J. C. (1959). *Acta bot. neerl.* **8**, 490–491.
White, N. H. (1954). *Aust. J. Sci.* **17**, 18–19.
Wilhelm, S. (1956). *Phytopathology*, **46**, 293–295.
Wilkins, W. H. and Harris, G. C. M. (1946). *Ann. appl. Biol.* **33**, 179–188.
Willoughby, L. G. (1961). *Trans. Br. mycol. Soc.* **44**, 305–332.
Winston, P. W. (1956). *Ecology*, **37**, 120–132.
Witkamp, M. (1960). *Meded. Inst. toegep. biol. Onderz. Nat.* **46**, 1–51.
Witkamp, M. and van der Drift, J. (1961). *Pl. Soil*, **15**, 295–311.
Wood, F. A. and Wilcoxson, R. D. (1960). *Plant Dis. Reptr.* **44**, 594.
Wright, J. M. (1956a). *Ann. appl. Biol.* **44**, 461–466.
Wright, J. M. (1956b). *Ann. appl. Biol.* **44**, 561–566.
Yarwood, C. E. (1946). *Mycologia*, **38**, 346–348.
Zentmyer, G. A. (1961). *Science*, **133**, 1595–1596.